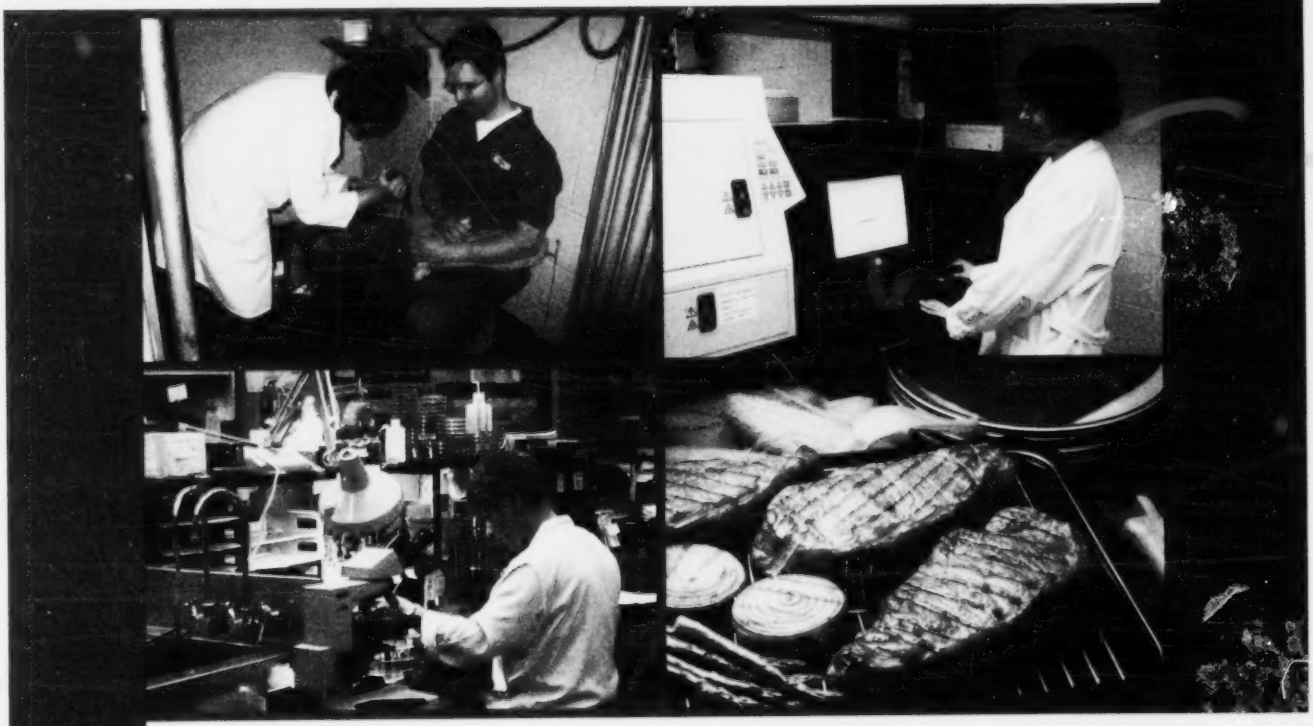
Ontario

Ontario Food Safety Research Program Compendium



Ontario Ministry of Agriculture and Food

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Cover photos:
Top left: Photo by Thomas Rosedal
Top right: Azadeh Namvar using image processing software employed in molecular typing studies.
Bottom left: Pat Weir performs a biochemical identification procedure

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Preface

The Ontario Government has long recognized the importance of food safety research as a tool to better characterize the risk presented by food contamination, to reduce risk through new detection instruments and methods, and to inform the development of effective regulations and policies. For this reason, the Food Safety Research Program (FSRP) was launched on February of 2001, as part of the Ontario Food Safety System, an initiative of three Ontario ministries: Ontario Ministry of Agriculture and Food (OMAF), Ministry of Natural Resources (MNR) and Ministry of Health and Long Term Care (MHLTC).

The FSRP was designed as an open, competitive, four-year, $4 million research fund with the purpose of enhancing food safety in Ontario through state-of-the-art research.

The main objectives of this program are:

- Development/validation of testing methods for the detection of pathogens and chemicals for use in laboratory and field settings;
- Identification of emerging hazards and contaminants;
- Risk analysis, risk assessment, risk management and control in food safety.

This is a report on the accomplishments of the Food Safety Research Program as we finalize the funding agreements for the fourth, and last, competition round. As you will see in the following pages, the FSRP has been remarkably successful in achieving not only its stated objectives, but also in fostering collaboration in food safety research, in providing a venue for researchers to discuss priorities and new ideas, and in disseminating information promptly and widely.

The FSRP is investing $4 million in 35 innovative projects from 11 research and development institutions, leveraging an additional $6 million through cash and in-kind contributions from our research partners. The program has contributed to the retention of highly qualified personnel by funding 62 post docs, Ph.D.s, masters and undergraduate students. This research has generated a large volume of new information and novel/improved technologies that are already being put into practice to improve the safety of our food supply.

The work reported here has been possible through the collective efforts of researchers, reviewers, universities, industry organizations, and private sector companies. To them, and to the staff of the Innovation and Risk Management Branch that administer the FSRP, we express our recognition and gratitude.

OMAF continues to consider food safety research as a fundamental and integral part of our on-going efforts to have one of the safest food systems in the world. We trust you will find the information on the FSRP and the projects abstracts useful.

Maurice Bitran, Ph.D.
Director
Innovation and Risk Management Branch
Ontario Ministry of Agriculture and Food

Gwen Zellen, Ph.D.
Director
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Ontario Ministry of Agriculture and Food

SECTION ONE: Food Safety Research Program

This Compendium provides information about 33 Food Safety Research Program research projects that have been approved for funding from fiscal year 2000/01 to 2003/04. Contracts for another two projects are under negotiations.

The abstracts are grouped in three categories: methodology development (MD); risk assessment (RA) and risk management (RM) in section two of the Compendium. Abstracts and impacts are condensed from proposals submitted by principal researchers who approved them prior to publication.

Research projects described in this Compendium cover many food safety aspects of the processes of food production from farm to fork - from the point at which food is grown or manufactured until it arrives on our plates. These projects target the development of advanced technologies and new knowledge that will help government regulators, industry, and consumers develop strategies to prevent, reduce, control or eliminate food safety risks. Applying risk assessment enables us to focus efforts on the highest-risk problems, resulting in a more effective use of resources.

This Compendium gives an account of the results of our research and how the research will help us achieve our objectives of protecting the health of the public and the interests of consumers in relation to food.

Background

Ontario is recognized throughout the world for the quality and safety of its agri-food products. To retain this position of leadership in food safety, the province has initiated science-based, field-to-fork Food Safety System improvements. In partnership with the Ministry of Health and Long-term Care (MOHLTC) and the Ministry of Natural Resources (MNR), the Ontario Ministry of Agriculture and Food (OMAF) led an Ontario food safety system review. During the review process, OMAF recognized the need to update its standards and requirements to keep pace with changes in scientific information, technology, consumer behavior, consumer lifestyles and industry practices. The review was designed to improve Ontario's food safety system by increasing the government's capacity to maintain high standards of food safety, protect public health and increase the marketability of Ontario food products. The overall goal is to build a:

- modernized, science-based food safety system founded on the principles of risk analysis/risk management;
- seamless system that covers the food chain from field to fork;
- market-friendly system consistent with Ontario's trade responsibilities and industry needs.

Program Overview

The program is open for competition among researchers from academia, industry, governmental agencies, private and regulatory labs, as well as partnerships of any of those. The researchers are eligible to apply for up to $250,000 per project that has to be completed within two years. The Innovation and Risk Management Branch of OMAF manages the program by facilitating identification and development of specific research priorities, by issuing an annual call for proposals, by facilitating an expert review process and by managing research contracts.

The mandate of the program is to support food safety research in three broad research areas:

- Development/validation of testing methods for the detection of pathogens and chemicals for use in laboratory and field settings;
- Identification of emerging hazards and contaminants;
- Risk analysis, risk assessment, risk management and control in food safety.

Every year the program has issued a Research Requirements Document (RRD) to invite eligible researchers to submit research proposals for funding. Three main research areas, as outlined in the RRD, were unchanged over the past four-year period. However, based on input from our stakeholders, a list of priorities within each main area changed from year to year to capture research trends and meet emerging food safety issues.

In addition, the RRD outlines the program policies and rules, including details about the review process. The proposal's fit to the research priorities described in the RRD, the relevance to current issues in food safety and the anticipated contribution to improving the food safety system in Ontario are some of the factors considered in the assessment of the proposals. To promote excellence in food safety research, the proposals are also peer-reviewed.

The progress of every funded project is monitored against a series of agreed milestones set out in the proposal and the program staff maintain regular contact with the researchers throughout the lifetime of a project. Upon completion, final project reports are reviewed and evaluated before being made available to the public, either through ministry avenues or publication in scientific literature. Once the projects are completed the actual impacts on Ontario's Food Safety System are assessed and communicated to OMAF's stakeholders.

OMAF is committed to ensuring the broad dissemination of research results. Information about the funded projects is available on the OMAF website as well as in this *Compendium*. Also, the successful applicants are required to register their project with the Inventory of Canadian Agri-food Research (*ICAR*). Project results are shared with government and industry representatives. Successful applicants are encouraged to publish their results in the appropriate journals, as well as to commercialize the inventions where possible.

Program Achievements

To date the program has completed four rounds of competition with 149 research proposals being submitted. A total of $4 million has been allocated to 35 projects, (Figure 1) that are in various stages of completion, at eleven research institutions in Ontario and Canada. The University of Guelph, Queen's University, University of Manitoba, University of Saskatchewan, Lakehead University, McMaster University, Ontario Beekeepers' Association, Agriculture and Agrifood Canada, and Health Canada are among the research institutions that have received funding under the program.

Descriptions of funded research projects are available from the web site:

http://www.gov.on.ca/OMAFRA/english/research/food/foodsafe.htm .

FSRP has funded 16 projects engaged in the development and/or validation of diagnostic methods (research area I - Figure 1) for rapid, accurate, reliable and less expensive detection of *E. coli*, *Salmonella*, *Campylobacter*, *M. paratuberculosis*, viruses and chemical residues (Figure 2) in food and water samples. The focus of some of the projects is on very advanced technology, such as development of microarrays and microchip technology for multi-pathogen detection in the same sample, or fiber optic technology for *E. coli* detection. In some cases the projects are focussed on improvements and validation of existing technology to improve turn around time or sample throughput.

A total of 11 projects are focussed on identification of emerging hazards and contaminants, risk assessment and/or data for risk assessment (research area II). Information on two of these projects is not available as the contracts are not signed. Once the contracts are signed, the information will be posted on OMAF's website. The projects that appear in this Compendium include:

- risk assessment of prevalence and antimicrobial resistance of *Campylobacter* in chicken, veal and turkey products at the retail level in Ontario;
- surveillance of Ontario pig farms for diseases of public health importance - with emphasis again on anitimicrobial resistance problems;
- the assessment of antimicrobial resistance in cull cows in Ontario dairy herds;
- implementation of HACCP-based food safety program implementation in the food service sector;
- transmission of pathogenic bacteria to horticulture crops via contaminated pesticide mixes;
- the investigation of routes for transfer of food and water-borne pathogens to produce - possible internalization of pathogens within produce edible tissue;
- the assessment of barriers to the effective implementation of HACCP in the Ontario food-processing sector.

Eight projects concentrate on development of strategies to eliminate, reduce, prevent and control food safety hazards (research area III); such as use of essential oils as an alternative to dietary antibiotics to control food-borne pathogens in livestock; use of ozonation to decontaminate horticulture produce; and the use of liquid feed in pork production - advantages and issues in terms of food safety.

Figure 1: # of projects submitted for funding vs. approved for funding (in total and by research area)

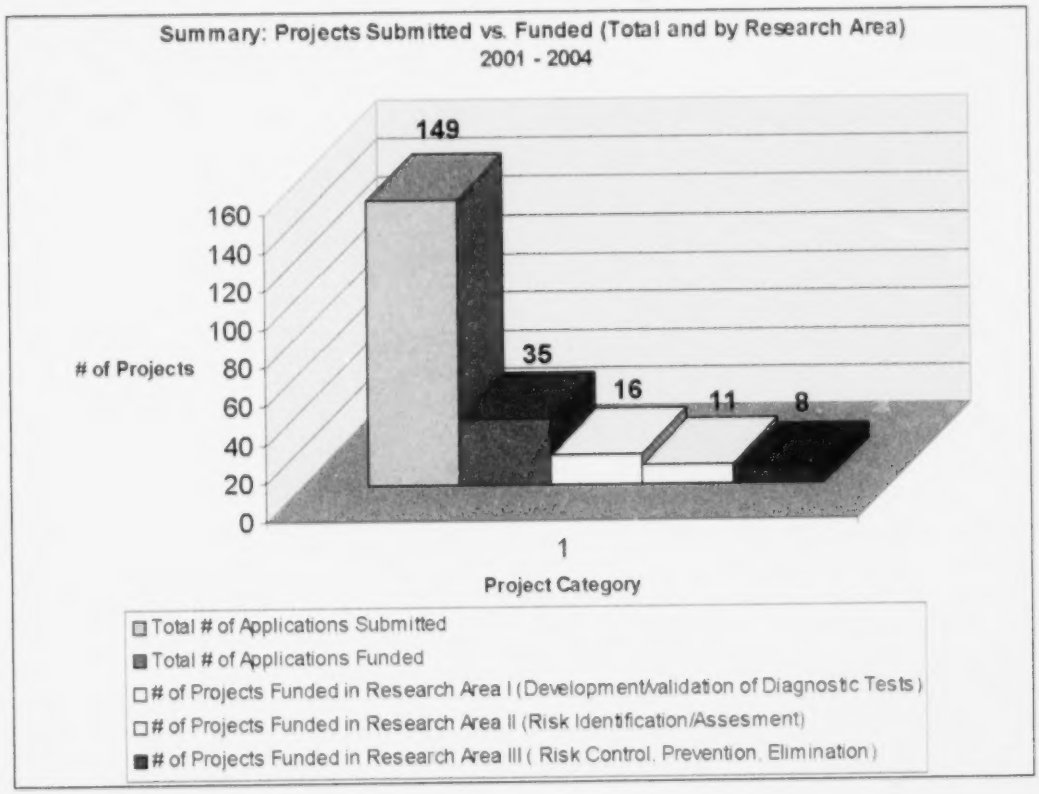
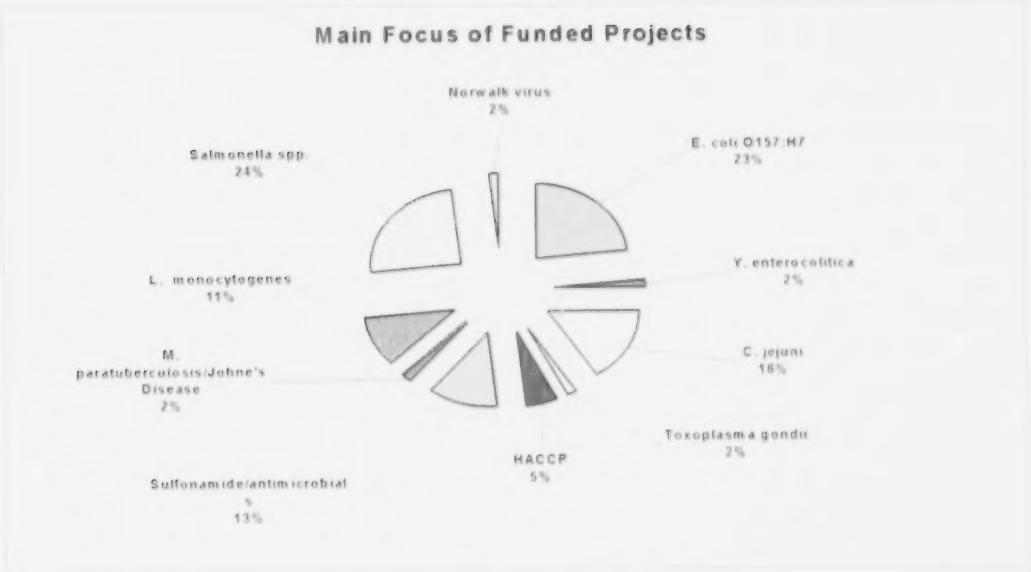


Figure 2: Main focus of funded projects



In addition, the investment in food safety research supports building the Ontario capacity in the food safety area - attraction and retention of diversified expertise. A total of 62 post doctoral, Ph.D., M.Sc., and B.Sc. students are supported through the program, which provides additional adequate expertise to support industry, government, academia, media, advisory panels, and expert committees needs in the food safety area.

Also, the program investment of $4 million dollars has leveraged over $6 million of additional investments by other provincial and federal governments and industry in food safety research in Ontario, thereby increasing the value of the program investment. The applicants' institutions contributed approximately $3.52 million dollars. An additional $2.6 (cash and in-kind) million dollars was provided by industry, other government agencies and universities. It is important to note that the program does not specifically require any matching funding.

Research supported through the FSRP generates new information, knowledge and technologies. These research outcomes help scientists carry out systematic assessments of risk to public safety, support the implementation of HACCP-based and quality assurance programs throughout the food chain and provide diagnostic tools to support field and laboratory testing for regulatory programs.

SECTION TWO: Abstracts

DM1: Johne's Disease - New Test Validation

Abstract:

Paratuberculosis (Johne's disease) is an infectious disease of ruminants caused by *Mycobacterium avium* spp *paratuberculosis* (*M. paratuberculosis*) considered to be a serious infectious disease currently plaguing the world's cattle industry. In the terminal stages of the disease, affected cows develop diarrhea and wasting. More importantly, most infected cows are asymptomatic but some will shed the organism in colostrum and milk as well as contaminate the environment by fecal shedding. In addition to animal health, a significant concern to consumers and to Ontario dairy producers is the controversial evidence that *M. paratuberculosis* may be causally linked with Crohn's disease of humans.

Control of Johne's disease has been complicated by the absence of rapid, cost-effective and sensitive individual or herd tests. Prevention would be much improved if diagnostic tests were able to determine the status of sub-clinical cows. The BACTEC culture system offers speed benefits over conventional culture. Also, IDEXX Laboratories recently launched an improved Johne's DNA probe for PCR.

The overall objective of this project is to provide rapid and accurate diagnostic tests to identify cattle infected with *M. paratuberculosis* in Ontario. The first goal is to set up and validate the BACTEC system and the IDEXX PCR to identify *M. paratuberculosis* in bovine feces. The last objective is to combine both procedures to reduce the turnaround time.

Expected Impact of Project Outcomes on Food Safety in Ontario:

This new testing methodology is now providing Ontario producers with rapid and accurate diagnostic tests for control programs for Johne's disease. In the past, fecal samples were sent to the USA for Johne's culture. Ontario now has the laboratory capability to culture *M. paratuberculosis* from diagnostic samples. Removal of infected animals will help eliminate the shedding of this pathogen into the environment and reduce the risk of human infection by *M. paratuberculosis* through milk, meat and water transfer. Control of *M. paratuberculosis* will also assist with domestic and international sales of cattle, especially to prominent US dairy states with active Johne's control programs.

Patricia Bell-Roger-molecular biology technologist
Jason Eidt-Mastitis, Johne's and molecular technologist

From left to right: bacteriology technologists Debbie Bateman- performing a Gram stain; Pat Weir- Biochemical identification procedure; Charles Doekes-Tissues set up procedure; Student-looking at bacterial colonies on agar plates.

Project Leader:
Marie Archambault, Ph.D.

Collaborating Researcher(s):
Grant Maxie, Hugh Cai, Dave Kelton, Davor Ojkic, Jocelyn Jansen, Steve Hendrick, Todd Duffield, Ken Leslie and Kerry Lissemore, University of Guelph

Project Duration:
Aug. 2001 - Jan. 2004

Contact information:
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SECTION TWO: Abstracts

DM1: Johne's Disease - New Test Validation

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Project Leader:

Collaborating Researcher(s):

Project Duration:

Contact information:

DM2: Development of a Rapid Microarray Diagnostic Assay for Detection of Norwalk-like Viruses in Food

Project Leader:
Sabah Bidawid, Ph.D.
Health Canada

Collaborating Researcher(s):
Michael Doyle,
University of Georgia;
Mansel Griffiths,
University of Guelph

Project Duration:
Apr. 2003 - Mar. 2005

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Abstract:

The proposed research aims at developing rapid viral concentration and nucleic acid extraction and detection techniques. Two potential virus concentration methods; adsorption-elution-precipitation and/or elution-precipitation will be investigated to concentrate viruses from selected foods. Furthermore, DNA microarrays will be developed for the sensitive detection of virus particles in the selected types of foods. The ultimate goal of this work is the development of a rapid and sensitive diagnostic tool to screen foods (and water as applicable) for the presence of food-borne viruses.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The development of novel, genetic technologies for virus detection will result in a marked improvement in the ability to detect, investigate, and control enteric food-borne viral outbreaks and related disease in Ontario. Furthermore, the development of rapid methods will aid in the evaluation and implementation of appropriate strategies to control viral contamination of foods, particularly in view of the fact that food-borne viruses are resistant to many of the traditional methods used to control bacterial pathogens. These rapid and sensitive methods will improve our surveillance capabilities, allow for developing a comprehensive database of domestically prevalent and imported strains of these viruses, provide tools for risk assessment, and would aid in the determination of regulatory options for monitoring food safety and epidemiological surveillance. This would allow the integration of a true farm-to-fork food safety approach for the management and control of viral food-borne disease in Ontario and across Canada.

Hybridization station: Automatised station which performs complete hybridization and washing protocols as defined by the user.

One wing in the Virology Laboratory leading to three separate molecular and cell culture workstation rooms.

Microarrayer: Robotic device that spots DNA fragments from a source plate onto specific areas on a glass slide to manufacture a microarray chip.

DM3: Development of a Rapid, Sensitive and Reliable Test for the Detection and Quantification of *Escherichia coli* in Foods

Abstract:

The goal of this project is to develop a rapid and sensitive, yet simple and reliable test for the identification and quantification of *E. coli* in foods.

The test is a modified version of the standard *E. coli* test where a sample is incubated for up to 24 hours in a solution containing a fluorogenic substrate. *E. coli* bacteria in the sample will grow and produce the enzyme ß-glucuronidase, which then hydrolyzes the substrate to produce a fluorescent product. The new fiber-optic test system uses a custom designed substrate compound and a fiber-optic probe designed to detect the unique product generated when *E. coli* consumes the substrate. A prototype commercial instrument has been constructed, consisting of a portable, computer controlled electro-optics unit and a small heated sample compartment.

Fluorescence is continually monitored, providing the earliest possible indication of a positive sample. Human judgment of the fluorescence is not required, and the probe can extract the optical signal even from coloured and turbid samples. In addition, by tracking the fluorescence signal over time, an estimate of the number of cells present in the initial sample can be made, providing a quantitative test. The method has been confirmed in milk and juice, and for vegetable washes which gave an "overgrown" result with conventional membrane filter tests. Highly contaminated samples can be confirmed positive in as little as 1-2 hours.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Current programs to enhance food safety, from HACCP to new regulations, always include a discussion of increased monitoring for pathogen indicators. This novel method has a potential for on-line screening of food and water samples, including source waters and ingredients, waste streams and final products. This can provide improved capacity for HACCP monitoring and regulatory screening programs.

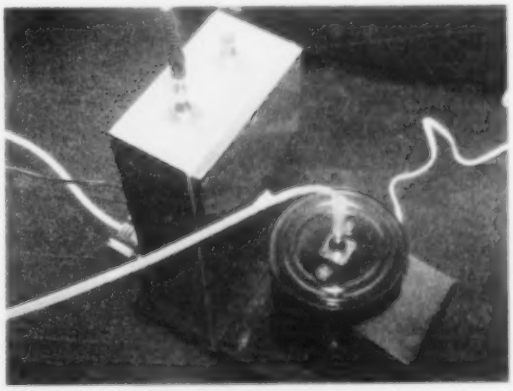

Photo-electronic unit with fiber-optic probe connected to heated sample chamber for detection of *E. coli*. Photo by Eric Marcotte

Project Leader:
Stephen Brown, Ph.D.
Queen's University

Collaborating Researcher(s):
Andrew Kropinski,
Queen's University

Project Duration:
Oct. 2001 - Oct. 2003

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DM2: Development of a Rapid Microarray Diagnostic Assay for Detection of Norwalk-like Viruses in Food

Project Leader:
Sabah Bidawid, Ph.D.
Health Canada

Collaborating Researcher(s):
Michael Doyle
University of Georgia
Marsel Griffiths,
University of Guelph

Project Duration:
Apr. 2003 - Mar. 2005

Abstract:

The proposed research aims at developing rapid and cost-effective and nucleic acid extraction and detection techniques. Two potential pre-concentration methods adsorption-elution precipitation and/or elution-precipitation will be investigated to concentrate viruses from selected foods. Furthermore, DNA microarrays will be developed for the sensitive detection of virus particles in the selected types of foods. The ultimate goal of this work is the development of a rapid and sensitive diagnostic tool to screen foods (and water as applicable) for the presence of food-borne viruses.

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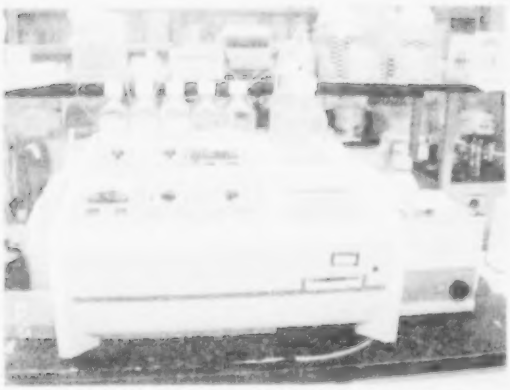
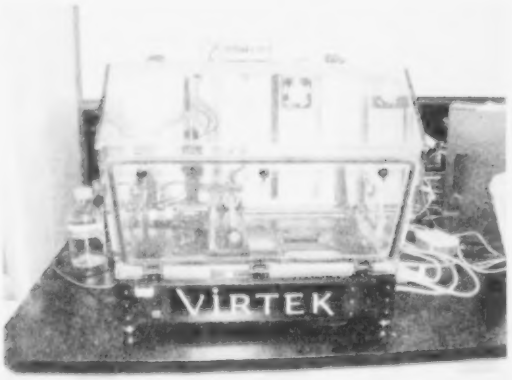


Hybridization station: Automatised station which performs complete hybridization and washing protocols as defined by the user.



Microarrayer: Robotic device that spots DNA fragments from a source plate onto specific areas on a glass slide to manufacture a microarray chip.

One wing in the Virology Laboratory leading to three separate molecular and cell culture workstation rooms.

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DM3: Development of a Rapid, Sensitive and Reliable Test for the Detection and Quantification of *Escherichia coli* in Foods

Abstract:

The goal of this project is to develop a rapid and sensitive, yet simple and reliable test for the identification and quantification of *E. coli* in foods.

The test is a modified version of the standard *E. coli* test where a sample is incubated for up to 24 hours in a solution containing a fluorogenic substrate. *E. coli* bacteria in the sample will grow and produce the enzyme ß-glucuronidase, which then hydrolyzes the substrate to produce a fluorescent product. The new fiber-optic test system uses a custom designed substrate compound and a fiber-optic probe designed to detect the unique product generated when *E. coli* consumes the substrate. A prototype commercial instrument has been constructed, consisting of a portable, computer controlled electro-optics unit and a small heated sample compartment.

Fluorescence is continually monitored, providing the earliest possible indication of a positive sample. Human judgment of the fluorescence is not required, and the probe can extract the optical signal even from coloured and turbid samples. In addition, by tracking the fluorescence signal over time, an estimate of the number of cells present in the initial sample can be made, providing a quantitative test. The method has been confirmed in milk and juice, and for vegetable washes which gave an "overgrown" result with conventional membrane filter tests. Highly contaminated samples can be confirmed positive in as little as 1-2 hours.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Current programs to enhance food safety, from HACCP to new regulations, always include a discussion of increased monitoring for pathogen indicators. This novel method has a potential for on-line screening of food and water samples, including source waters and ingredients, waste streams and final products. This can provide improved capacity for HACCP monitoring and regulatory screening programs.

Photo electronic unit with fiber-optic probe connected to heated sample chamber for detection of *E. coli*. Photo by Eric Marcotte

Project Leader:
Stephen Brown, Ph.D.
Queen's University

Collaborating Researcher(s):
Andrew Krepinski,
Queen's University

Project Duration:
Oct. 2001 - Oct. 2003

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DM4: Improvement and Validation of a DNA Microchip-Based Test for Rapid and Simultaneous Detection of Six Food-Borne Pathogens in Food Samples

Project Leader:
Shu Chen, Ph.D.
University of Guelph

Collaborating Researcher(s):
Joseph Odumeru, Laboratory Services Division, University of Guelph;
Roger Johnson and Kris Rahn, Laboratory for Foodborne Zoonoses, Health Canada;
David Chiu, Department of Computing and Information Science, University of Guelph

Project Duration:
Aug. 2001 - Jan. 2003

Abstract:

Current diagnostic assays only allow for detection of a single pathogen at a time in food samples. However, there are a number of food-borne pathogens that are today's major public concerns. The DNA chip or microarray-based technology allows for simultaneous detection of large numbers of pathogens in a single sample. In previous projects, a prototype microarray-based test was developed for simultaneous detection of six common food-borne bacterial pathogens in a single test, including Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* serotype O157:H7, *Salmonella*, *Salmonella typhimurium* DT104, *Listeria monocytogenes* and *Campylobacter*. The purpose of this project was to improve, optimize and validate the prototype microchip and associated procedures so that the chip-based test can eventually be used in diagnostic settings.

The system was improved, optimized and validated for its specificity using 771 target and 248 non-target microorganisms. The microchip results demonstrated correct identification of all the organisms tested. The detection limits of the system were 50 -1000 CFU/mL of pre-enrichment broth when spiked milk, lettuce, ground beef and ground pork samples were used. The system was also evaluated using 1012 pre-enriched food broths that were derived from 512 naturally contaminated foods, and 40 fecal samples. The percentages of correlation between cultural and the PCR-microchip methods for positive samples were 77% for *Campylobacter*, 91% for *Salmonella*, 91% for *L. monocytogenes* and 87% for *E. coli*, and for negative samples were 97%, 84%, 85% and 90% for the four organisms respectively. In addition, customized software was created to facilitate data calculation, interpretation and reporting.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The project resulted in a chip-based test and its associated software that can potentially be used for rapid, specific and simultaneous detection of six food-borne pathogens in a single test in food samples. The availability of such a test will allow diagnostic labs to provide faster and cost-effective results to agri-food industries and consumers and provide an avenue to enhance the safety of Ontario's food supplies. This will also allow testing laboratories and government inspection staff to conduct risk assessment studies in a cost-effective and systematic manner in support of monitoring and surveillance activities and enforcement of regulatory programs.

Microarray Scanner. Laboratory Services, University of Guelph. Photo by Dan Vautour.

Senior Molecular Biologist, Dr. Jiping Li, is analyzing microarray results. Laboratory Services, University of Guelph. Photo by Dan Vautour.

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DM5: Development of a Novel Protein Chip-Based Test for Rapid and Cost-effective *Salmonella* Serotyping

Abstract:

Salmonella is one of the most important agents of food-borne illnesses. Serotyping is the most important typing method for characterization of *Salmonella* isolates, and involves the immunological classification of two surface structures, lipopolysaccharide (O antigens) and Phase 1 and Phase 2 flagella antigens, based on the Kauffmann-White scheme. This scheme employs more than 250 antisera for identification of over 2500 *Salmonella* serotypes. The current *Salmonella* serotyping method does not allow detection of multiple antigens simultaneously, and requires a minimum of three antibody-antigen reactions to identify a particular serovar. It takes at least two to three days to provide an answer, and is available mainly in reference laboratories. The objective of this study is to develop a rapid, cost-effective and easy-to-use alternative assay for *Salmonella* serotyping based on the protein microarray technology.

In this project, methods have been established to immobilize *Salmonella* antibodies onto the glass slides, to label *Salmonella* cells directly with a fluorescent dye (Eosin Y or Cy3), and to capture and specifically detect the labeled *Salmonella* cells with the antibody array. A model antibody (6x6) array was constructed for identification of *Salmonella* serotypes: Enteritidis, Heildelberg and Typhimurium. The model array was able to detect *Salmonella* O antigens, and Phase 1 and Phase 2 flagella antigens simultaneously, and correctly identify the three serotypes when it was tested using 10 *Salmonella* reference strains. The model array has been expanded for typing 20 commonly-identified and clinically-important *Salmonella* serotypes. The expanded array is being optimized. The specificity and sensitivity of the array will be determined using a limited number of *Salmonella* and non-*Salmonella* bacteria, and verified with the standard Kauffmann-White typing scheme.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Successful completion of the project will result in a rapid, simple, accurate and cost-effective protein chip based-test that can be used for serotyping of commonly-identified and clinically important *Salmonella* serovars upon further validation. The availability of such a test will eventually allow diagnostic labs, such as Laboratory Services Division, University of Guelph, to provide faster and cost-effective results to food and animal industries and government agencies, and to improve the efficiency and effectiveness in providing greater services to Ontario's agri-food in the areas of food safety and animal disease control.

Microarray Team. Laboratory Services, University of Guelph. Left to right: Ms. Mary Murphy, Dr. Shu Chen, Ms. Emily Goulden, Ms. Liuqiong Lu, Dr. Jiping Li. Photo by Donna Foran.

Project Leader:
Shu Chen, Ph.D.
University of Guelph

Collaborating Researcher(s):
Hugh Cai, Laboratory Services Division, University of Guelph;
Anne Muckle, Laboratory for Foodborne Zoonoses, Health Canada;
John Prescott, Department of Pathobiology, University of Guelph

Project Duration:
Jun. 2002 - May 2004

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DM4: Improvement and Validation of a DNA Microchip-Based Test for Rapid and Simultaneous Detection of Six Food-Borne Pathogens in Food Samples

Project Leader:
Shu Chen, Ph.D.
University of Guelph

Collaborating Researcher(s):
Joseph Odumeru, Laboratory Services Division, University of Guelph;
Roger Johnson and Kris Ralm, Laboratory for Foodborne Zoonoses, Health Canada;
David Chiu, Department of Computing and Information Science, University of Guelph

Project Duration:
Aug. 2001 - Jan. 2003

Abstract:

Current diagnostic assays only allow for detection of a single pathogen at a time in food samples. However, there are a number of food-borne pathogens that are today's major public concerns. The DNA chip or microarray based technology allows for simultaneous detection of large numbers of pathogens in a single sample. In previous projects, a prototype microarray-based test was developed for simultaneous detection of six common food-borne bacterial pathogens in a single test, including Shiga toxin-producing *Escherichia coli* (STEC), *E. coli* serotype O157:H7, *Salmonella*, *Salmonella typhimurium* DT104, *Listeria monocytogenes* and *Campylobacter*. The purpose of this project was to improve, optimize and validate the prototype microchip and associated procedures so that the chip-based test can eventually be used in diagnostic settings.

The system was improved, optimized and validated for its specificity using 771 target and 248 non-target microorganisms. The microchip results demonstrated correct identification of all the organisms tested. The detection limits of the system were 50 -1000 CFU/mL of pre-enrichment broth when spiked milk, lettuce, ground beef and ground pork samples were used. The system was also evaluated using 1012 pre-enriched food broths that were derived from 512 naturally contaminated foods, and 40 fecal samples. The percentages of correlation between cultural and the PCR-microchip methods for positive samples were 77% for *Campylobacter*, 91% for *Salmonella*, 91% for *L. monocytogenes* and 87% for *E. coli*, and for negative samples were 97%, 84%, 85% and 90% for the four organisms respectively. In addition, customized software was created to facilitate data calculation, interpretation and reporting.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The project resulted in a chip-based test and its associated software that can potentially be used for rapid, specific and simultaneous detection of six food-borne pathogens in a single test in food samples. The availability of such a test will allow diagnostic labs to provide faster and cost-effective results to agri-food industries and consumers and provide an avenue to enhance the safety of Ontario's food supplies. This will also allow testing laboratories and government inspection staff to conduct risk assessment studies in a cost-effective and systematic manner in support of monitoring and surveillance activities and enforcement of regulatory programs.

Microarray Scanner, Laboratory Services, University of Guelph. Photo by Dan Vautour.

Senior Molecular Biologist, Dr. Jiping Li, is analyzing microarray results. Laboratory Services, University of Guelph. Photo by Dan Vautour.

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DM5: Development of a Novel Protein Chip-Based Test for Rapid and Cost-effective *Salmonella* Serotyping

Abstract:

Salmonella is one of the most important agents of food-borne illnesses. Serotyping is the most important typing method for characterization of *Salmonella* isolates, and involves the immunological classification of two surface structures, lipopolysaccharide (O antigens) and Phase 1 and Phase 2 flagella antigens, based on the Kauffmann-White scheme. This scheme employs more than 250 antisera for identification of over 2500 *Salmonella* serotypes. The current *Salmonella* serotyping method does not allow detection of multiple antigens simultaneously, and requires a minimum of three antibody-antigen reactions to identify a particular serovar. It takes at least two to three days to provide an answer, and is available mainly in reference laboratories. The objective of this study is to develop a rapid, cost-effective and easy-to-use alternative assay for *Salmonella* serotyping based on the protein microarray technology.

In this project, methods have been established to immobilize *Salmonella* antibodies onto the glass slides, to label *Salmonella* cells directly with a fluorescent dye (Eosin Y or Cy3), and to capture and specifically detect the labeled *Salmonella* cells with the antibody array. A model antibody (6x6) array was constructed for identification of *Salmonella* serotypes: Enteritidis, Heildelberg and Typhimurium. The model array was able to detect *Salmonella* O antigens, and Phase 1 and Phase 2 flagella antigens simultaneously, and correctly identify the three serotypes when it was tested using 10 *Salmonella* reference strains. The model array has been expanded for typing 20 commonly-identified and clinically-important *Salmonella* serotypes. The expanded array is being optimized. The specificity and sensitivity of the array will be determined using a limited number of *Salmonella* and non-*Salmonella* bacteria, and verified with the standard Kauffmann-White typing scheme.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Successful completion of the project will result in a rapid, simple, accurate and cost-effective protein chip based-test that can be used for serotyping of commonly-identified and clinically important *Salmonella* serovars upon further validation. The availability of such a test will eventually allow diagnostic labs, such as Laboratory Services Division, University of Guelph, to provide faster and cost-effective results to food and animal industries and government agencies, and to improve the efficiency and effectiveness in providing greater services to Ontario's agri-food in the areas of food safety and animal disease control.

Microarray Team, Laboratory Services, University of Guelph. Left to right: Ms. Mary Murphy, Dr. Shu Chen, Ms. Emily Goulden, Ms. Luqiong Lu, Dr. Jiping Li. Photo by Donna Foran.

Project Leader:
Shu Chen, Ph.D.
University of Guelph

Collaborating Researcher(s):
Hugh Cai, Laboratory Services Division, University of Guelph;
Anne Muckle, Laboratory for Foodborne Zoonoses, Health Canada;
John Prescott, Department of Pathobiology, University of Guelph

Project Duration:
June 2002 - May 2004

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DM6: Development of a Robust DNA Preparation Method to Enhance Simultaneous Detection of Multiple Pathogens in Foods by a Microarray-based Assay

Project Leader:
Shu Chen, Ph.D.
University of Guelph

Collaborating Researcher(s):
Joseph Odumeru, Laboratory Services Division, University of Guelph

Project Duration:
Nov. 2003 - Jun. 2005

Abstract:

The purpose of this project is to develop a robust DNA preparation method that can be integrated into the Lab existing multiplex PCR/microarray-based assay for simultaneous detection of nine bacterial and parasitic pathogens in foods in a single test. The proposed method is based on the new multiple displacement amplification (MDA) technology that employs ϕ 29 DNA polymerase and exonuclease-resistant primers for faithful replication of the original DNA population. Procedures for DNA isolation and enrichment by MDA will be developed for various foods. It will be explored whether the MDA method can be directly used with microarray without PCR.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The deliverable of the project will be a robust DNA extraction and enrichment method for food samples, including poultry, red meat, milk and vegetables, which can be used as a front end for PCR/microarray-based assays. The availability of such a DNA preparation method (a front end) will allow diagnostic labs to apply the powerful microarrays in food testing to provide faster and more cost-effective results to food industries and government agencies and provide an avenue to enhance the safety of Ontario's food supplies.

This will also allow scientists to conduct risk assessment studies in a systematic and cost-effective manner in support of monitoring and surveillance activities and enforcement of regulatory programs. This will help to ensure that the Ontario agri-food industries remain competitive in providing safe foods to today's food safety conscious consumers.

The Molecular Biology Lab, Laboratory Services, University of Guelph where a number of food and water safety related microarray research projects have been conducted. Research Scientist, Dr. Shu Chen is analyzing microarray data. Photo by Dan Vautour.

Microarray Team. Laboratory Services, University of Guelph. Left to right: Ms. Jennifer Brisbin, Ms. Emily Goulden, Dr. Jiping Li, Dr. Shu Chen, Ms. Liuqiong Lu, Dr. Hugh Cai and Dr. Shukui Guan. Photo by Dan Vautour.

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DM7: New Technologies for Improving Real - Time PCR Methods for Detection of Food-borne Pathogens

Abstract:

The aim of this project is to develop real-time PCR systems which integrate sample preparation, signal amplification and data handling to produce an analytical system enabling Ontario agencies to meet the anticipated demands for rapid and reliable detection of microorganisms, including *Listeria* spp., *Salmonella* spp., *Campylobacter jejuni*, *E. coli* O157:H7 and total *E. coli* in a variety of food, water, and environmental samples. The methods will meet regulatory standards for the detection and enumeration of target organisms in time frames ranging from a few hours to overnight.

Expected Impact of Project Outcomes on Food Safety in Ontario:

A successful completion of this project will allow the following:

- Improved sample perpetration, signal amplification and data handling to enable an analytical system for microbial detec tion.
- Automated analysis (30 minutes) with semi-quantitative measurements of a number of food-borne pathogens.
- Simple, reliable and rapid detection kit for detection of *Salmonella* spp. *E. coli* O157:H7, *E. coli* Biotype I, *Listeria monocytogenes* and *Campylobacter jejuni*.

Birce Mercanoglou uses the Lightcycler at the Canadian Research Institute for Food Safety to detect *Salmonella* in foods.

Project Leader:
Mansel Griffiths, Ph.D.
University of Guelph

Collaborating Researcher(s):
R. Johnson, Health Canada, Guelph;
B.W. Blais, CFIA, Ottawa;
D. H. Evans and L. Brovko, University of Guelph

Project Duration:
Jan. 2003 - Dec. 2004

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DM6: Development of a Robust DNA Preparation Method to Enhance Simultaneous Detection of Multiple Pathogens in Foods by a Microarray-based Assay

Project Leader:
Shu Chen, Ph.D.
University of Guelph

Collaborating Researcher(s):
Joseph Odumeru, Laboratory Services Division, University of Guelph

Project Duration:
Nov. 2003 - Jun. 2005

Abstract:

The purpose of this project is to develop a robust DNA preparation method that can be integrated into the Lab-on-a-chip multiplex PCR-microarray-based assay for simultaneous detection of key bacteria and parasitic pathogens in foods in a single test. This protocol testing is based on the new multiple displacement amplification (MDA) technology that employs ϕ 29 DNA polymerase and exonuclease-resistant random primers for faithful replication of the original DNA population. The results for DNA isolation and enrichment by MDA will be examined for various foods. It will be explored whether the MDA method can be directly used for microarray analysis.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The deliverable of the project will be a robust DNA extraction and enrichment method for food samples, including poultry, red meat, milk and vegetables, which can be used as a front end for PCR-microarray-based assays. The availability of such a DNA preparation method (a front end) will allow diagnostic labs to apply the powerful microarrays in food testing to provide faster and more cost effective results to food industries and government agencies and provide an avenue to enhance the safety of Ontario's food supplies.

This will also allow scientists to conduct risk assessment studies in a systematic and cost-effective manner in support of monitoring and surveillance activities and enforcement of regulatory programs. This will help to ensure that the Ontario agri-food industries remain competitive in providing safe foods to today's food safety conscious consumers.

The Molecular Biology Lab, Laboratory Services, University of Guelph where a number of food and water safety related microarray research projects have been conducted. Research Scientist, Dr. Shu Chen is analyzing microarray data. Photo by Dan Vautour.

Microarray Team, Laboratory Services, University of Guelph. Left to right: Ms. Jennifer Brisbin, Ms. Emily Goulden, Dr. Jiping Li, Dr. Shu Chen, Ms. Liuqiong Lu, Dr. Hugh Cai and Dr. Shukun Guan. Photo by Dan Vautour.

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DM7: New Technologies for Improving Real - Time PCR Methods for Detection of Food-borne Pathogens

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Project Leader:
Mansel Griffiths, Ph.D.
University of Guelph

Collaborating Researcher(s):
R. Johnson, Health Canada, Guelph;
B.W. Blais, CFIA, Ottawa;
E. H. Evans and L. Brooks, University of Guelph

Project Duration:
Jan. 2003 - Dec. 2004

Birce Mercanoglou uses the Lightcycler at the Canadian Research Institute for Food Safety to detect *Salmonella* in foods.

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DM8: Rapid Phage-based Method for the Detection of Pathogens in Food

Project Leader:
Mansel Griffiths, Ph.D.
University of Guelph

Collaborating Researcher(s):
L. Brovko and
L. Goodridge,
University of Guelph

Project Duration:
Oct. 2003 - Dec. 2005

Abstract:

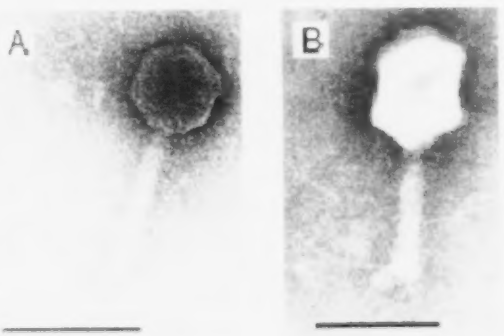
The goal of the project is to develop and evaluate a bacteriophage-based system for the simultaneous capture and detection of foodborne pathogens in food and environmental samples. The system will use immobilized host-specific reporter bacteriophage to concentrate the target bacteria on the surface of magnetic beads and, following bacterial infection by the phage, a bioluminescent or fluorescent signal will appear with intensity proportional to the initial number of bacteria in the sample.

For constructing reporter bacteriophages, two different reporter systems will be used. In the first, lux genes will be introduced into the phage genome using protocols described previously. After the infection the developing bioluminescence will be monitored and correlation between initial cell number and in vivo bioluminescence will be established. The second reporter phage will be constructed by fusion of GFP gene with phage head protein gene, thus providing release of phage particles labeled with GFP after infection. Immobilization of phages will be performed by introduction of biotin or biotin-binding peptides onto the bacteriophage head. This will be done both by chemical modification of amino- or carboxyl-groups of head or by fusion of head protein with the biotin-binding peptide according to a published experimental protocol. Biotinylated bacteriophages will be immobilized on streptavidin-coated solid surface resulting in formation of a biosorbent.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The test developed from this project will enable processors and regulatory agencies to quickly establish whether a specific pathogen is present in food and provide an estimate of the level of contamination.

It is anticipated that that this technology will allow the detection of a single bacterial cell in a sample within 2-3 hours and this can be achieved using simple equipment available even in field conditions.



Transmission electron micrographs of bacteriophages LG1 (A) and AR1 (B). Bars, 100 nm. (From Goodridge et al. 2003 Applied and Environmental Microbiology 69:5364).

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DM9: Validation of a Method for Determining the Species of Origin of Contaminant *E. coli*

Abstract:

The goal of this research is to provide a validated procedure whereby *E. coli* isolated from an environmental source can be assigned to a human, wildlife, or livestock origin. This study is based on work that has recently been completed by the project researchers. That research evaluated four methods for determining the sources from which *E. coli* isolates came. The study showed that a nucleic acid-based method called Amplified Fragment Length Polymorphism (AFLP) was highly effective and was the best of the four methods tested. Because four methods were being evaluated, a limited number of *E. coli* isolates was investigated by the AFLP procedure. Furthermore, the studies were not conducted in a blinded manner.

This study extends the previous study by conducting the tests on *E. coli* isolated from environmental samples as well as from animals and humans; and by conducting the tests in a double blind fashion and requiring the procedure to identify the source.

Expected Impact of Project Outcomes on Food Safety in Ontario:

There is increasing concern with the problem of contamination of water sources with fecal material of undetermined origin. The study will aid in identifying the sources of *E. coli* contamination, thereby enabling remedial steps to be undertaken.

Project Leader:
Carlton Gyles, Ph.D.
University of Guelph

Collaborating Researcher(s):
J. Odumeru and S. Chen, Laboratory Services Division, University of Guelph

Project Duration:
Nov. 2003 - Apr. 2004

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DM8: Rapid Phage-based Method for the Detection of Pathogens in Food

Project Leader:

Collaborating Researcher(s):

Project Duration:

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Contact information:

DM9: Validation of a Method for Determining the Species of Origin of Contaminant *E. coli*

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Project Leader:

Collaborating Researcher(s):

Project Duration:

Contact information:

DM10: Fluorescence Polarization Immunoassays (FPIA) for Food Safety: a Rapid Detection System for Pathogens and Chemicals

Project Leader:
Christopher Hall, Ph.D.
University of Guelph

Collaborating Researcher(s):
Kurt Almoquist, University of Guelph; Klaus Nielson, CFIA; Roger McKenzie, NRC; Mick Jolley, Diachemix. Inc.

Project Duration:
Nov. 2003 - Oct. 2005

Abstract:

Fluorescence Polarization Immunoassays (FPIAs) are rapid, accurate and homogeneous. Fluorescent-labeled small molecules rotate rapidly in solution and when bound to larger molecules (i.e. antibodies), the rotation rate is decreased and measurable. FP readers are available in high-throughput-screening (HTS) formats (>50,000 samples/day) or as field portable instruments.

FPIA offers competitive advantages over existing technologies because they are quicker, easier, portable, and inexpensive. Most FP tests can be performed in minutes and are considered to be equivalent or superior in performance to enzyme-linked immunosorbent assays (ELISAs) and high performance liquid chromatography (HPLC).

This study seeks to expand the utility of food/water-borne pathogen/chemical-specific antibodies for rapid, accurate and simple use in both laboratory and field settings by formatting them as fluorescence polarization immunoassays.

Expected Impact of Project Outcomes on Food Safety in Ontario:

FPIAs offer competitive advantages over existing technologies because they are rapid, easier, portable, and less expensive to perform. Most FP tests can be performed in seconds to minutes and are considered to be equivalent or superior in performance to Enzyme Linked Immunosorbent Assays (ELISAs) and are much more portable and amenable to high throughput screening than ELISA, HPLC, and GC/MS analysis.

Multiple testing platforms are available. A one-well fluorescence polarization diagnostic instrument has been developed for field use. This instrument is lightweight and portable, with no moving parts and minimal power requirements provided from a laptop computer battery. For high throughput screening (HTS) applications several FP instruments (i.e. the EnVision™ in our laboratory) can perform up to 50,000 samples per day. This method, based on ease of use and versatility, would be available as an inexpensive and rapid platform technology for adoption and use by the food and water safety network in Ontario.

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DM11: *Campylobacter* Isolation Methodology and Molecular Characterization

Abstract:

There are two main objectives of this project. The first objective is to further investigate the relationship between the *Campylobacter* spp. isolated from the human and chicken samples utilizing molecular characterization techniques. A subset of human and poultry isolates with similar antimicrobial resistance profiles will be evaluated using PCR-DNA sequence analysis of a short variable region within the *fla* gene. This will provide more complete and definitive information for the analysis of the relationship between antimicrobial resistance in retail poultry and human isolates and the risk factors associated with human campylobacteriosis.

The second objective is to investigate and compare the performance of the current method for enumeration of *Campylobacter* spp. from carcass rinse samples with the Campy-Cefex Agar method from both carcass rinse samples and package weep fluid samples. The Campy-Cefex Agar method is a faster and more economical method than is currently utilized in research situations. The additional advantage of the package weep fluid sample is the ease of sample collection and the non-destructive nature of the sampling. Enumeration of *Campylobacter* spp. in retail poultry provides information on the level of risk to which the consumer is exposed. This information is an important component of risk analysis and risk assessments. Currently, enumeration is not practical for inclusion in surveillance or the majority of research projects due to the extensive resource and time commitments of the official method.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The results of this project will contribute to the maintenance of Ontario's standards of food safety and preservation of public health by: providing a rapid and inexpensive method for enumeration of *Campylobyacter* in meat; improve general understanding of the potential transmission of antimicrobial resistant stains of *Campylobacter* via the food chain; and provide the basis for further initiatives including investigations into the association between on-farm antimicrobial use and the occurrence of antimicrobial resistant strains of *Campylobacter* in retail poultry.

Project Leader:
Scott McEwen, Ph.D.
University of Guelph

Collaborating Research Establishment(s):
Laboratory for Foodborne Zoonoses, Health Canada; Division of Enteric, Foodborne and Waterborne Diseases, Bureau of Infectious Diseases, Health Canada; Clinical and Environmental Microbiology Department, Central Public Health Laboratory, Health Services Division, Ontario Ministry of Health and Long-Term Care

Project Duration:
Oct. 2001 - Jul. 2004

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DM10: Fluorescence Polarization Immunoassays (FPIA) for Food Safety: a Rapid Detection System for Pathogens and Chemicals

Project Leader:
Christopher Hall, Ph.D
University of Guelph

Collaborating Researcher(s):
Kurt Almquist, University of Guelph
Klaus Nielson, CFIA
Roger McKenzie, NRC
Mick Jolley, Diachemix Inc.

Project Duration:
Nov. 2003 - Oct. 2005

Abstract:

Fluorescence Polarization Immunoassays (FPIAs) are rapid, accurate and homogenous. Fluorescent-labeled small molecules rotate rapidly in solution and when bound to larger molecules (i.e. antibodies), the rotation rate is decreased and measurable. FP readers are available in high throughput screening (HTS) formats (> 50,000 samples/day) or as hand portable instruments.

FPIA offers competitive advantages over existing technologies because they are quicker, easier, portable, and inexpensive. Most FP tests can be performed in minutes and are considered to be equivalent or superior in performance to enzyme-linked immunosorbent assays (ELISAs) and high performance liquid chromatography (HPLC).

This study aims to expand the utility of food- and water-borne pathogen/chemical specific antibodies for rapid, accurate and simple use in both laboratory and field settings by formatting them as fluorescence polarization immunoassays.

Expected Impact of Project Outcomes on Food Safety in Ontario:

FPIAs offer competitive advantages over existing technologies because they are rapid, easier, portable, and less expensive to perform. Most FP tests can be performed in seconds to minutes and are considered to be equivalent or superior in performance to Enzyme Linked Immunosorbent Assays (ELISAs) and are much more portable and amenable to high throughput screening than ELISA, HPLC, and GC/MS analysis.

Multiple testing platforms are available. A new well fluorescence polarization diagnostic instrument has been developed for field use. This instrument is lightweight and portable with no moving parts and minimal power requirements provided from a laptop computer battery. For high throughput screening (HTS) applications, several FP instruments (i.e. the Envision) in our laboratory can perform up to 50,000 samples per day. This method, based on ease of use and versatility, would be available as an alternative and rapid platform technology for adoption and use by the food and water safety network in Ontario.

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DM11: *Campylobacter* Isolation Methodology and Molecular Characterization

Abstract:

There are two main objectives of this project. The first objective is to further investigate the relationship between the *Campylobacter* spp. isolated from the human and chicken samples utilizing molecular characterization techniques. A subset of human and poultry isolates with similar antimicrobial resistance profiles will be evaluated using PCR-DNA sequence analysis of a short variable region within the *flaA* gene. This will provide more complete and definitive information for the analysis of the relationship between antimicrobial resistance in retail poultry and human isolates and the risk factors associated with human campylobacteriosis.

The second objective is to investigate and compare the performance of the current method for enumeration of *Campylobacter* spp. from carcass rinse samples with the Campy-Cefex Agar method from both carcass rinse samples and package weep fluid samples. The Campy-Cefex Agar method is a faster and more economical method than is currently utilized in research situations. The additional advantage of the package weep fluid sample is the ease of sample collection and the non-destructive nature of the sampling. Enumeration of *Campylobacter* spp. in retail poultry provides information on the level of risk to which the consumer is exposed. This information is an important component of risk analysis and risk assessments. Currently, enumeration is not practical for routine surveillance or for the majority of research projects due to the extensive resource and labor requirements of the official method.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The results of this project will contribute to the maintenance of Ontario's standards of food safety and preservation of public health by providing a rapid and inexpensive method for enumeration of *Campylobacter* in meat, improve general understanding of the potential transmission of antimicrobial resistant strains of *Campylobacter* via the food chain and provide the basis for further research including investigations into the association between on-farm antimicrobial use and the occurrence of antimicrobial resistant strains of *Campylobacter* in retail poultry.

Project Leader:
Scott McEwen, Ph.D.
University of Guelph

Collaborating Research Establishment(s):
Laboratory for Foodborne Zoonoses, Health Canada; Division of Enteric, Foodborne and Waterborne Diseases, Bureau of Infectious Diseases, Health Canada; Clinical and Environmental Microbiology Department, Central Public Health Laboratory, Health Services Division, Ontario Ministry of Health and Long-Term Care

Project Duration:
Oct. 2001 - Jul. 2004

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DM12: Development and Validation of a Screening Protocol for Identifying Sulfamethazine-Violative Swine Carcasses at Ontario Abattoirs Using the CHARM ROSA™ Sulfamethazine Test

Project Leader:
Mark Mitchell
University of Guelph

Collaborating Researcher(s):
Louise Spilsbury, Laboratory Services Division, University of Guelph

Project Duration:
Jan. 2002 - Oct. 2003

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Abstract:

The CHARM ROSA™ (Rapid One Step Assay) Sulfamethazine (SMZ) Test for milk was adapted to urine and compared to the Sulfa On Site Test (SOS) to examine the suitability of using the ROSA as an alternative to the SOS. The SOS is a thin layer chromatographic method widely used in North American abattoirs for screening porcine urine samples for SMZ residues. The ROSA sulfamethazine test is a lateral flow test strip currently marketed for screening milk. A suitable urine dilution buffer and protocol was developed to adapt the ROSA test to urine testing. Two dilutions were determined to adjust test sensitivity to urine target levels, 1:150 for 0.4 ppm SMZ (corresponding to violative SMZ levels in the liver tissue) and 1:600 for 1.3 ppm SMZ (corresponding to violative SMZ levels in muscle tissue). The concentration response of the dilution schemes were determined using fortified urine samples and demonstrated 90% sensitivity (ie. positive) with 95% confidence via probit analysis at the 0.252 ppm and 1.033 ppm SMZ urine levels. Meat inspectors at an Ontario abattoir field tested a two-tiered ROSA testing model that screened at the 0.4 ppm level, for condemnation of violative offal, with subsequent confirmation of positive urines at the 1.3 ppm level to predict tissue positive carcasses. Results were compared to the SOS test results of 294 urine samples. Comparative tables showed that relative to the SOS test the ROSA test had sensitivity and negative predictive values of 100% at the 0.4 ppm and 1.3 ppm SMZ target levels. The test specificity (ie. negative) was 86% at the 0.4 ppm target level and 96% at the 1.3 ppm target level. TLC/densitometry confirmatory analysis of 1:600 dilution positive samples found liver violative levels in 2 samples, non-violative levels in 13 samples and not detected in 1 sample. TLC/densitometry on 13 of the 16 corresponding positive muscle tissue samples
found SMZ residues at violative levels in 1 sample, non-violative levels in 8 samples and not detected in 4 samples. These results indicate that the CHARM ROSA™ SMZ test using a 1:600 dilution may be reliably used for screening porcine urine for SMZ residues in abattoirs. Greater dilutions of urine may be necessary to more closely predict organ and tissue violative levels.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The application of the new method will eliminate the need to hold non-violative carcasses overnight or longer while waiting for laboratory test results, thereby freeing up storage space at the abattoirs and hastening the transfer of product to market. It will demonstrate environmental responsibility and concern for HACCP violations by removing the need for a laboratory method that utilizes toxic solvents requiring expensive disposal practices, as well as the use of glass capillary tubes. The new method is much simpler to perform decreasing the chance for testing errors.

New sulfa testing method set up at one of the abattoirs in Ontario. Photo taken by Mike Eastment, OMAF, Meat Inspection Branch.

DM13: Application of the Impedance and Colorimetric Systems for Rapid and Cost Effective Detection of *Listeria* Species in Food and Environmental Samples

Abstract:

Listeria monocytogenes, the pathogenic species of *Listeria* has been shown to form biofilms in various food-processing surfaces. Biofilm growth of *L. monocytogenes* provides a substantial risk of pathogen contamination especially if wet surfaces are not maintained in a sanitary condition. Many food and meat processors test environmental swabs and sponges to confirm the absence of *Listeria* species, as part of their GMP and HACCP verification programs. Culture method, the most commonly used method, requires up to seven days to complete and does not fit well into these programs which require immediate remedial action.

This study will be assessing the application of two semi-automated systems, impedance-based system (Malthus) and a colorimetric-based system (Biosys) for rapid and low-cost detection of *Listeria* species, in food and environmental samples. The swab and sponge samples collected from a food processing plant which monitors for *Listeria* spp., specifically *L. monocytogenes* and *L. innocua*, the two most prevalent listerias in food processing environments will be tested.

Expected Impact of Project Outcomes on Food Safety in Ontario:

A validated Impedance and Colorimetric-based screening system for the detection of *Listeria* spp. in food and environmental samples which allows detection results within 24-30 hours of testing, as opposed to 6-7 days of testing by culture-based methods. This automated alternative reduces the cost of testing by 50%.

Dr. Odumeru and his team are working with a Micro-Foss-Biosys instrument.

Project Leader:
Joseph Odumeru, Ph.D.
University of Guelph

Collaborating Researcher(s):
Ruth Eden, Biosys Inc,;
Gerald Moore, Medox Diagnostics Inc.;
Marcia Maldonado, Pride Pak Ltd.

Project Duration:
Oct. 2001 - Feb. 2004

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DM12: Development and Validation of a Screening Protocol for Identifying Sulfamethazine-Violative Swine Carcasses at Ontario Abattoirs Using the CHARM ROSA™ Sulfamethazine Test

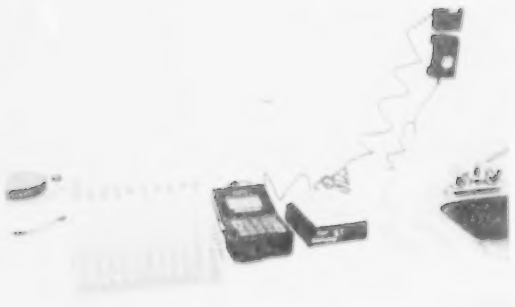
Project Leader:
Mark Mitchell
University of Guelph

Collaborating Researcher(s):
Louise Sandberg
Laboratory Services Division, University of Guelph

Project Duration:
Jan. 2002 - Oct. 2003

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:



New sulfa testing method set up at one of the abattoirs in Ontario. Photo taken by Mike Eastment, OMAF, Meat Inspection Branch.

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DM13: Application of the Impedance and Colorimetric Systems for Rapid and Cost Effective Detection of *Listeria* Species in Food and Environmental Samples

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Dr. Odumeru and his team are working with a Micro Foss Biosys instrument.

Project Leader:

Collaborating Researcher(s):

Project Duration:

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DM14: Evaluation of Immunoassay Based Kits with High Sensitivity and Specificity for Rapid Detection of *E. coli* O157:H7 in Foods

Project Leader:
Joseph Odumeru, Ph.D.
University of Guelph

Collaborating Researcher(s):
Carlos Leon-Velarde,
University of Guelph

Project Duration:
Sept. 2002 - Aug. 2003

Abstract:

In this study, an evaluation of the sensitivity and specificity of detection of the ImmunoCard STAT! *E. coli* O157:H7 assay will be conducted on a variety of food matrices commonly associated with *E. coli* O157:H7 contamination. This detection technique incorporates monoclonal antibodies against the O157 and H7 antigens and provides a possible solution for reducing the occurrence of false positives while maintaining a high sensitivity of detection.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The outcome of this study will be validated, culture-based, rapid, sensitive and specific screening method for *E. coli* O157:H7 detection in food samples. The ability of such assay to specifically detect *E. coli* O157:H7 within 24 hours of testing would provide a rapid turn around time for reporting test results as well as reducing the occurrence of false positives and misidentification, while maintaining a high sensitivity of detection. Also, rapid cultural isolation of *E. coli* O157:H7 from presumptive positive samples is essential not only for confirmatory purposes, but also to provide unequivocal results to regulatory agencies that require an isolate for further strain characterization in an outbreak investigation.

Dr. Odumeru is working on the improvement of methods for pathogen detection.

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DM15: Detection of *Campylobacter jejuni* by Fluorescent *in Situ* Hybridization

Abstract:

Hazard Analysis Critical Control Point (HACCP) systems require verification procedures that include confirmation of the presence of pathogens in finished product and processing environments. *C. jejuni* is a microaerophilic organism that requires specialized culture conditions for growth. This involves the use of special gaseous atmospheres and culture supplements. Thus, only a few specialized laboratories can currently analyze food and environmental samples for *C. jejuni* and testing tends to be very expensive. This significantly impedes monitoring and surveillance of *C. jejuni* in poultry production.

This project will use the molecular biology approach known as fluorescent *in situ* hybridization to develop a rapid, culture independent procedure for the detection of *C. jejuni* in poultry and in environmental samples from poultry processing environments. This approach will overcome the difficulties associated with culturing while still being specific for viable *C. jejuni*. The work will be completed in three milestones: first, the specificity of an existing fluorescent *in situ* hybridization (FISH) protocol will be confirmed using a wide range of *C. jejuni* isolates and non-*Campylobacter* isolates. Second, a buoyant centrifugation based procedure will be adopted which will allow to concentrate *C. jejuni* from large sample volumes and to separate microbial cells from particles that can interfere with FISH detection. Third, the newly established FISH protocol will be tested and validated with spiked and naturally contaminated poultry products and environmental samples from processing environments.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The results of this study will support poultry producers and processors in their ongoing efforts of reducing prevalence of the pathogen in the production chain and provide regulatory labs with a cost-effective procedure for surveillance and enforcement programs.

Project Leader:
Heidi Schraft, Ph.D.
Lakehead University

Project Duration:
May 2002 - Apr. 2004

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DM14: Evaluation of Immunoassay Based Kits with High Sensitivity and Specificity for Rapid Detection of *E. coli* O157:H7 in Foods

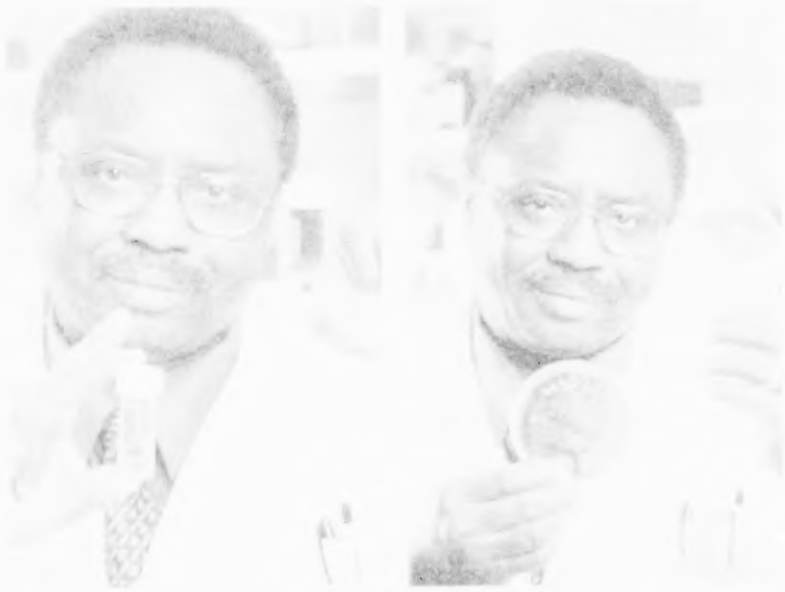
Project Leader:
Joseph Odumeru, Ph.D.
University of Guelph

Collaborating Researcher(s):
Carlos Leon-Velarde
University of Guelph

Project Duration:
Sept. 2002 - Aug. 2003

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:



Dr. Odumeru is working on the improvement of methods for pathogen detection.

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DM15: Detection of *Campylobacter jejuni* by Fluorescent *in Situ* Hybridization

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Project Leader:

Project Duration:

Contact information:

DM16: Development of an Analytical Method for the Confirmation of Sulfonamides in Animal Tissues

Project Leader:
Louise Spilsbury
University of Guelph

Project Duration:
May 2001 - Sept. 2003

Abstract:

The objective of this project is to develop and validate a High Performance Liquid Chromatography (HPLC) method for the determination of sulfonamide residues in animal tissues.

A method has been developed for the detection and quantitation of eight sulfonamide residues in swine muscle and liver. Sulfamonomethoxine or sulfamethoxypyridazine is added to the tissue as an internal standard to correct for variations in recovery. Sample clean-up begins with homogenization with sodium sulfate and ethyl acetate, followed by centrifugation and removal of the solvent by evaporation. The residue is redissolved in an ethyl acetate-hexane solution, and loaded on to cation exchange solid phase extraction columns. The sulfonamides are eluted with a buffer-acetonitrile solution. An aliquot is derivatized with fluorescamine and 25 μL injected on to the HPLC. Separation is by reversed-phase HPLC with a gradient mobile phase consisting of acetate buffer and acetonitrile. The calibration curves for the sulfonamides cover the range of 0.05 ppm to 0.2 ppm ($r \geq 0.995$). Limit of detection is 0.005 ppm and limit of quantitation is 0.015 ppm or better. Repeatability at 0.1 ppm ranges from 6.6% to 13.3%.

Expected Impact of Project Outcomes on Food Safety in Ontario:

This method offers several advantages. It uses no halogenated solvents, which are toxic and expensive to dispose of. It removes the need to maintain an aging and obsolete scanning densitometer that has limited application. The Ontario food system will benefit from the wider range of sulfonamide residues capable of being detected and quantified in the meat of food animals. It is anticipated that the method will be readily adaptable to other matrices, such as meat-and-bone meal, and feed.

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RA1: Identification of Bacterial Components That Influence Colonization of Poultry by *Campylobacter jejuni*

Abstract:

Campylobacter jejuni is the leading cause of bacterial gastroenteritis in humans in North America. The handling and consumption of poultry has been identified as a major risk factor for infection by *C. jejuni*. Chicks, at one-day of age, are not colonized by *C. jejuni*, but by two weeks of age it is possible to detect colonization of birds. At time of slaughter, between 30 and 100% of the flocks are colonized. During the slaughter process *C. jejuni* may be spread to previously uncontaminated carcasses. Colonization of poultry produces no harmful effect in the birds. Reduction of the level of colonization of poultry has been identified as a priority to increase food safety by the industry.

The objective of this study is to investigate factors which contribute to the colonization of poultry by *C. jejuni*, as well as that are important in the spread of *C. jejuni* from one animal to another.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Reduction of the level of colonization of poultry by *C. jejuni* is a priority of the poultry industry in Ontario and Canada. The organisms are common in the environment and this makes exclusion by use of biosecurity alone generally unfeasible. The efficiency by which *C. jejuni* colonizes poultry is very strain dependent. The results of this study will greatly improve a complete understanding of the process of the colonization of poultry by *C. jejuni*, which will facilitate the development of control strategies to prevent this problem and enhance food safety.

Project Leader:
Brenda Allan, Ph.D.
University of Saskatchewan

Collaborating Researcher(s):
K. Amoako, VIDO, Saskatoon;
C. Szymanski, NRC

Project Duration:
Nov. 2002 - Oct. 2004

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DM16: Development of an Analytical Method for the Confirmation of Sulfonamides in Animal Tissues

Project Leader:

Project Duration:

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Contact information:

RA1: Identification of Bacterial Components That Influence Colonization of Poultry by *Campylobacter jejuni*

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Project Leader:

Collaborating Researchers:

Project Duration:

Contact Information:

RA2: Liquid Feeding of Swine - Potential Positive and Negative Impacts on Pork Safety

Project Leader:
C. F. M. de Lange, Ph.D.
University of Guelph

Collaborating Researcher(s):
R. Friendship, H. Namkung, E. Jjearond, J. Squire, [1]University of Guelph;
J. Gong, Agriculture & Agri-Food Canada

Project Duration:
Sept. 2002 - Dec. 2004

Abstract:

Recent studies suggest that liquid feeding, and especially feeding of fermented feeds, can enhance gut health in pigs, reduce the reliance on in-feed medication in commercial pork production and reduce the contamination of pork products with *Salmonella* spp. In Ontario a wide range of by-products from the food industry is currently used, or considered for use, in liquid feeding of swine. This study will survey types and suppliers of liquid swine feed ingredients in Ontario, including a characterization of pathogens (especially *Salmonella* spp.) and harmful chemicals in these ingredients and develop a suggested blueprint for routine ingredient evaluation. In addition, the study will characterize changes in microflora, especially the development of pathogens, during fermentation of selected liquid swine feed ingredients, with and without incubating the fermentation medium with probiotics. An impact of feeding non-medicated fermented feed to growing pigs on growth performance, gut health, fecal excretion of pathogens and contamination of pig carcasses and pork with pathogens and harmful chemicals will be established.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The generated information will allow for more accurate and objective assessment of positive and negative impacts of liquid feeding of swine on safety of Ontario pork products. Moreover, critical control points in pork production and strategies to enhance safety of Ontario pork products will be identified.

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RA3: Surveillance of Ontario pig farms for diseases of public health significance

Abstract:

A sample of approximately 100 pig farms representing the wide spectrum of operations in Ontario were involved in this study. Farms were visited at least 3 times over a 2 year time span, and at each visit, blood samples were taken from 30 sows and 30 finisher hogs and 15 manure samples collected from finisher pigs. A management-housing survey was performed at the time of the visit. This information included details about drug use. The samples were used to examine for the presence of micro-organisms or the presence of antibodies to micro-organisms that are of interest from a public health standpoint.

From manure samples, the following disease causing organisms were identified by culture or other means: *Salmonella* sp., *E. coli* O157:H7, *Yersinia enterocolitica*, *Balantidium coli*, *Giardia* sp. and *Cryptosporidia*. From blood samples, the following diseases were evaluated by serological tests measuring the presence of antibodies: *Salmonella* sp. Swine Influenza virus (including H_1N_1, H_3N_2 Colorado and Texas strains), and *Toxoplasma gondii*. For each pathogen, there were at least a few farms with positive results. This was the first time in North America that *E. coli* O157:H7 was identified from pig manure.

The presence and prevalence of these diseases were determined and the data were analyzed to evaluate risk factors. For example, the presence of cats on a farm was strong risk factor for herds to be positive for Toxomplasmosis, and the use of all-in/all-out management by site appeared to be associated with a lower risk of finding *Yersinia enterocolitica*.

More than half the producers participating in this study were using antibiotics as growth promoters in the grower-finisher ration.

Non-pathogenic *E. coli* that were tested for antimicrobial resistance demonstrated that multiple drug resistance was common but unrelated to on-farm drug use. Importantly, no resistance was found (from the more than 600 isolates tested) against the newer and very important antibiotics like 3rd generation cephalosporins and fluoroquinolones.

Expected Impact of Project Outcomes on Food Safety in Ontario:

This project has provided information on the presence and prevalence of diseases of public health significance which will allow the industry to plan strategies for controlling these potential problems. Where risk factors have been identified, programs can be implemented to reduce or eliminate the problem. For example, Toxoplasmosis was identified as a problem on a small number of farms. The implementation of a ban on cats in pig barns and feed preparation areas would eliminate this health risk.

If the Ontario industry was to implement an on-going monitoring program with financial rewards or penalties, this project provides a baseline which can be used to determine factors such as number of samples needed to be collected and which tests to use. One conclusion from this work is that further refinement on testing procedures is required. The sensitivity and specificity of many of the tests is less than ideal or not fully understood.

Project Leader:
Robert Friendship, Ph.D.
University of Guelph

Collaborating Researcher(s):
C. Dewey, Department of Population Medicine, University of Guelph;
S. McEwen, Department of Population Medicine, University of Guelph;
C. Gyles, Department of Pathobiology, University of Guelph

Project Duration:
Jul. 2001 - Jul. 2003

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RA2: Liquid Feeding of Swine - Potential Positive and Negative Impacts on Pork Safety

Project Leader:

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

RA3: Surveillance of Ontario pig farms for diseases of public health significance

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

RA4: Investigation of Routes for Transfer of Food and Water-borne Pathogens to Produce

Project Leader:
Mansel Griffiths, Ph.D.
University of Guelph

Collaborating Researcher(s):
L. Brovko,
University of Guelph

Project Duration:
Oct. 2002 - Dec. 2003

Abstract:

This project seeks to determine if some common food-borne pathogens, *Salmonella* spp., *E. coli* O157:H7 and *Listeria monocytogenes*, when present in manure or irrigation water applied to soil invade the tissues and fruit of lettuce, tomatoes, strawberries and cucumbers. In addition, the effect of root damage and the presence of known plant pathogens in the soil on the ability of the food-borne bacteria to enter the plant will be determined. This study builds upon the two recent studies that have demonstrated the ability of *E. coli* O157:H7 to enter lettuce tissues when introduced into soil by irrigation water or manure.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The results of this study will provide an answer whether *Salmonella*, *E.coli* O157:H7, *Listeria monocytogenes* from contaminated soil, irrigated water and manure can enter the plant tissue and internalize them. This will allow identification of risks associated with the presence of pathogens in soil and will help identify strategies to control these hazards.

Scanning electron micrographs of cress roots from Day 8 seedlings derived from seeds inoculated with *Escherichia coli* O157:H7 Ph1. Photograph provided by J. Jablasone, K. Warriner, and M.W. Griffiths.

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RA5: Evaluation of Pesticide Solutions in the Transmission of Pathogenic Bacteria to Horticultural Crops

Abstract:

This study is based on a premise that introduction of bacterial pathogens such as *Salmonella*, *Listeria*, *E. coli* and *Shigella* by liquid pesticides applied to horticultural crops, could increase the risk of food-borne illness to individuals who consume such products. An overall objective of this study is to evaluate the potential of food safety associated risks with the spraying of pesticides, prepared using on-farm water of unknown bacteriological quality, onto horticultural crops.

The study will involve a laboratory assessment of the ability of *Salmonella*, *Listeria*, *E. coli* or *Shigella* to grow in selected, recommended pesticides used on Ontario horticultural crops, and conditions affecting growth of pathogens in pesticides: time, temperature, concentration of pesticide (water volume), and presence of competing microorganisms. A small plot field trial will be carried out with the objective to assess the potential risk of applying pesticides if pathogen growth has occurred, one pesticide shown to promote pathogen growth will be inoculated with a mixture of two pathogens, and applied onto a horticultural crop (e.g. tomatoes).

Expected Impact of Project Outcomes on Food Safety in Ontario:

The outcomes of this research will allow for identification of possible risk factors associated with the use (including storage) of on-farm water (especially surface waters) in mixing pesticides applied to commercial fruit and vegetable crops. They also will enhance knowledge and scientific information pertaining to the identification of on-farm critical control points and a contribution of information to pesticide education programs.
In addition, pesticides requiring special handling or caution with respect to mixing and storing will be identified, as well as future research needs in this area.

Project Leader:
Richard Holley, Ph.D.
University of Manitoba

Collaborating Researcher(s):
Greg Blank, G. Crow, University of Manitoba; Mansel Griffiths, University of Guelph

Project Duration:
Sept. 2002 - Mar. 2004

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RA4: Investigation of Routes for Transfer of Food and Water-borne Pathogens to Produce

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

RA5: Evaluation of Pesticide Solutions in the Transmission of Pathogenic Bacteria to Horticultural Crops

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Project Leader:
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Collaborating Researcher(s):
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Project Duration:
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RA6: Assessing the Incidence of Antimicrobial Resistant *E. coli* and *Salmonella* Bacterial Isolates in Cull Cows from Ontario Free Stall Dairy Herds

Project Leader:
David Kelton
University of Guelph

Collaborating Researcher(s):
K. Lissemore, S. Martin, S. McEwen,
University of Guelph;
R. Reid-Smith,
Health Canada

Project Duration:
Oct. 2001 - Oct. 2003

Abstract:

The objective of this project is to determine the incidence of antimicrobial resistant *E. coli* and *Salmonella* spp. in cull dairy cows entering the food system as beef using an incidence density case-control observational study design. A second objective is to identify key aspects of facility design and cow management that are associated with herd level antimicrobial drug use and the incidence of resistance development.

Surveys assessing various aspects of antimicrobial use will be mailed to all producers with free-stall dairy barns and all livestock veterinarians in the province of Ontario. Additionally, 25 free-stall herds are currently enrolled in a longitudinal study in which producers track individual and herd level drug use for a one year period using a paper-based treatment record and a garbage can auditing (GCA) system. Cull cows from the study herds will be assessed with regard to previous exposure to antimicrobial drugs and fecal samples will be collected and cultured for *E. coli* and *Salmonella* isolates will be typed. The incidence of resistant isolates in cull cows (cases) will be determined and compared to the incidence in cows remaining in the herd (controls). Cow and herd level data will be collected to be included as predictor variables in a model that will assess the association of the incidence of antimicrobial drug resistant *E. coli* and *Salmonella* in cull cows with specific risk factors.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Results of this project will provide information of the incidence of antimcrobial resistant *E. coli* and *Salmonella* spp. in cull dairy cows entering the food system as beef.

Key aspects of facility design and cow management that are associated with herd level antimicrobial drug use and the incidence of resistance development will be also determined.

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RA7: Occurrence of Enteric Pathogens and Antimicrobial Resistance Patterns in Selected Retail Poultry Products and Human Cases in a Southwestern Ontario County

Abstract:

This project is an investigation into the occurrence of *Campylobacter* spp. in retail poultry in a county in southwestern Ontario, the occurrence of human campylobacteriosis, the risk factors associated with human campylobacteriosis, and the antimicrobial resistance patterns of poultry and human isolates.

The objectives of this study are: to investigate and compare the occurrence of susceptible and resistant *Campylobacter* spp. isolates in retail poultry and human cases of campylobacteriosis in a defined geographical area in Ontario; to investigate the risk factors for, and burden of illness resulting from, susceptible and resistant campylobacteriosis; and to investigate the temporal relationship between prevalence of *Campylobacter* contamination in poultry and the occurrence of campylobacteriosis.

The study will document and characterize the occurrence of susceptible and resistant strains of *Campylobacter* spp. in retail poultry over a one year period in a defined geographical area (subject county/Health Unit). Poultry samples will be purchased at pre-determined intervals from retail outlets according to a defined sampling frame and protocol. *Campylobacter* isolates will be cultured and serotyped according to standard methodology and resistance tested using the E-test system. For the same time period, the stool samples of all diagnosed human cases of campylobacteriosis will be processed according to like protocols. Epidemiological data will be collected from cases and a randomly selected comparison group. The correlation between the resistance patterns observed in the poultry isolates and the human isolates, the temporal relationship between the level of *Campylobacter* isolated from retail poultry and the occurrence of campylobacteriosis, and the risk factors for the occurrence of susceptible and resistant campylobacteriosis will be analyzed.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The results of this project will contribute to the maintenance of Ontario's standards of food safety and preservation of public health by: providing preliminary Ontario-based prevalence and risk factor data; providing direction for future larger scale research needed to produce the type of representative data needed for meaningful assessments; provide preliminary data for the development of HACCP-based quality assurance programs; improve our understanding of the potential transmission of antimicrobial resistant strains of *Campylobacter* via the food-chain; and provide the basis for further initiatives including investigations into the association between on-farm antimicrobial use and the occurrence of antimicrobial resistant strains of *Campylobacter* in retail poultry and the development of an active surveillance system for antimicrobial resistant foodborne pathogens in Ontario.

Project Leader:
Scott McEwen, Ph.D.
University of Guelph

Collaborating Researcher(s):
A. Deckert, R. Reid-Smith, R. Irwin, A. Valdivieso, K. Dore, P. Buck, Health Canada; F. Jamieson and B. Ciebin, Ontario Ministry of Health and Long-Term Care

Project Duration:
Jun. 2001 - Jan. 2004

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RA6: Assessing the Incidence of Antimicrobial Resistant *E. coli* and *Salmonella* Bacterial Isolates in Cull Cows from Ontario Free Stall Dairy Herds

Project Leader:

Collaborating Researcher(s):

Project Duration:

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Contact information:

RA7: Occurrence of Enteric Pathogens and Antimicrobial Resistance Patterns in Selected Retail Poultry Products and Human Cases in a Southwestern Ontario County

Project Leader:
Scott McEwen, Ph.D.
University of Guelph

Collaborating Researcher(s):
A. Deckert, R. Reid-Smith, R. Irwin, A. Valliyeso, K. Dore, P. Buck, Health Canada
F. Jamieson and B. Clarke, Ontario Ministry of Health and Long-Term Care

Project Duration:
Jan. 2001 - Jan. 2004

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

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RA8: Occurrence of Enteric Pathogens and Antimicrobial Resistance Patterns in Selected Retail Turkey and Veal Products in Southwestern Ontario

Project Leader:
Carl Ribble, Ph.D.
University of Guelph

Collaborating Researcher(s):
Scott McEwen, University of Guelph; R. Reid-Smith, Rebecca Irwin, Anne Deckert, Alfonso Valdivieso and Cornelius Poppe, LFZ, Health Canada, Guelph

Project Duration:
Sept. 2002 - Nov. 2004

Abstract:

This project is an investigation into the occurrence and antimicrobial resistance patterns of *Campylobacter*, *Salmonella* and *E. coli* in retail turkey and veal in three southwestern Ontario counties. Samples will be purchased at predetermined intervals from retail outlets according to a defined sampling frame and protocol. Isolates will be cultured and serotyped (*Salmonella*) according to standard methodology and tested for antimicrobial resistance using the E-test (*Campylobacter*) and Sensititre (*Salmonella* and *E. coli*) systems.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The results of this study will contribute to the maintenance of Ontario's standards of food safety and preservation of public health by:

- Providing preliminary Ontario-based prevalence data -for use in risk assessments and the development of on-farm HACCP-based programs.
- Improving knowledge of the potential transmission of antimicrobial resistant strains of *Campylobacter*, *Salmonella* and *E. coli* via the food chain.
- Providing a basis for further initiatives including investigations into the association between on-farm antimicrobial use and the occurrence of antimicrobial resistant bacteria contaminating retail turkey and veal and the development of an active surveillance system for antimicrobial resistant food-borne pathogens in Canada.

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RA9: Establishment of Critical Control Points for Enteric Pathogens in Beef Production

Abstract:

The association of virulent pathogens, such as *E. coli* O157:H7, with beef has provoked a need to improve food safety within the industry. To this end HACCP has been made mandatory in federal licensed slaughterhouses. However, the issue whether to implement HACCP in provincial plants is still cause for debate. Studies to date would suggest that current good manufacturing practice produces meat of comparable microbiological quality of that produced under HACCP. Given the range of slaughterhouse line speeds and capacities it is likely that the hazards in a large plant would be different to those in smaller operations. In the proposed study the carriage and transfer of enteric contamination in federal and provincial beef processing lines (farm to breaking) will be compared. Traditionally, verification studies have been based on indicator counts (total viable count, coliforms) or pathogen prevalence. Although plate count data can provide an overall assessment of the success of HACCP/GMP schemes it cannot indicate the origins of the contamination or key cross-contamination sites (Critical Control Points). In this study molecular typing of *E. coli* will be used to trace the carriage and transfer of enteric contamination on carcasses. In addition, detailed studies will be performed to establish the significance pre-slaughter handling and airborne contamination in defining the microbiological quality of beef carcasses.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Applying HACCP requires a significant input of resources and implementation would impose a burden on many small to medium plants. The key research question to be addressed in this proposal is to provide scientific data to determine the extent to which current good practice schemes operated by provincial slaughter lines provides an adequate protection against supplying meat contaminated with virulent pathogens. This will provide valuable data in supporting future food safety polices with regard regulating beef processing within Ontario.

Azadeh Namvar (MSc student, UofG) using image processing software employed in molecular typing studies.

Project Leader:
Keith Warriner, Ph.D.
University of Guelph

Collaborating Researcher(s):
S. Barbut and M. Griffiths, University of Guelph

Project Duration:
Jan. 2004 - Jun. 2005

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RA8: Occurrence of Enteric Pathogens and Antimicrobial Resistance Patterns in Selected Retail Turkey and Veal Products in Southwestern Ontario

Project Leader:

Collaborating Researcher(s):

Project Duration:

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

- Providing preliminary Ontario based prevalence data for use in risk assessments and the development of Ontario HACCP-based programs.
- Increasing knowledge of the potential transmission of antimicrobial resistant strains of *Campylobacter*, *Salmonella* and *E. coli* O157:H7 to the food chain.
- Providing a basis for further initiatives, including investigations into the association between on-farm antimicrobial use and the occurrence of antimicrobial resistant bacteria contaminating retail turkey and veal, and the development of an active surveillance system for antimicrobial resistant food borne pathogens in Canada.

Contact information:

RA9: Establishment of Critical Control Points for Enteric Pathogens in Beef Production

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Azadeh Namvar (MSc student, Uof G) using image processing software employed in molecular typing studies

RM1: A Community Trial to Determine an Effective Intervention for the Delivery of HACCP to the Food Service Sector

Project Leader:
Tom Abernathy, Ph.D.
Central West Planning Information Network - Hamilton

Collaborating Establishment(s):
Brant County Health Unit, Haldimand-Norfolk Regional Health Department, Halton Regional Health Department, City of Hamilton Social & Public Health Services Division, Regional Niagara Public Health Department, Waterloo Region Community Health Department, Wellington-Dufferin-Guelph Health Unit

Project Duration:
Nov. 2001 - Aug. 2003

Contact information:
Tom Abernathy
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Abstract:

Food-borne diseases are important contributors to human morbidity and mortality, and result in a significant cost to the health care sector. Recent changes in eating habits, and the tendency for people to eat more of their meals outside of the home, only increase the risk posed by these illnesses. One method for improving the operation of restaurants draws from the experience of the food processing industry. It involves the introduction of a system of procedures based on Hazard Analysis and the identification and modification of Critical Control Points (HACCP).

This project evaluated a program that applied the principles of HACCP to the food service industry. It successfully demonstrated that among a subpopulation of restaurant operations such a program not only is acceptable, but capable of producing tangible results. Results also suggest that the principles and methods of the program (i.e., an initial assessment of the site, working with the operator to identify and suggest improvements, and return visits to monitor compliance) could be transferred to other types of food service operations.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Results of this project will be shared with public health units in the province, operators of food service premises, the provincial government and the public health community through presentations, reports and published articles.

This project will contribute to the food safety system in Ontario by evaluating how the application of HACCP principles can be applied successfully to the food service industry. The result will be an improved food safety intervention to benefit those at the local, provincial, federal levels of government as well as the food service industry itself.

RM2: Evaluation of Essential Oils as an Alternative to Dietary Antibiotics to Control Food-borne Pathogens in Livestock

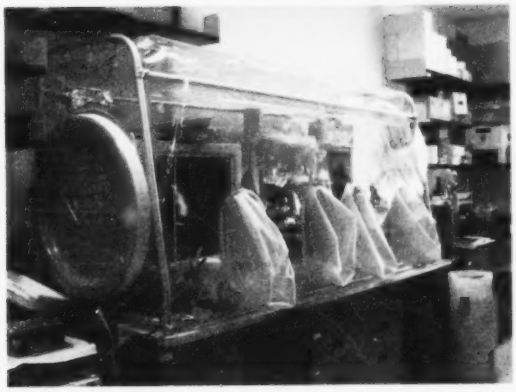
Abstract:

This study is to identify essential oils that can selectively inhibit the growth of *Salmonella* and *E. coli* O157:H7 *in vitro*. It will also examine the effects of identified essential oils on *Salmonella* and *E.coli* O157:H7, gut flora, gut health, and animal growth performance *in vivo*.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The success of this project would provide an alternative means to control *Salmonella* and *E. coli* O157:H7 in swine production. This could potentially reduce the reliance of the industry on dietary antibiotics, minimize the development of antibiotic resistance and enhance Ontario food safety and quality.

Dr. Joshua Gong, Research Scientist at AAFC with research interests in alternatives to dietary antibiotic.



Anaerobic chamber, oxygen-free atmosphere essential for culturing of gut bacteria and development of probiotics.

Project Leader:
Joshua Gong, Ph.D.
Agriculture and Agri-Food Canada

Collaborating Researcher(s):
R. Cao and T. Zhou, Food Research Program, AAFC; C. F. de Lange and R. Friendship, University of Guelph; C. Poppe and R. Johnson, LFZ, Health Canada, Guelph; W. Du, OMAR

Project Duration:
Nov. 2002 - Dec. 2004

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RM1: A Community Trial to Determine an Effective Intervention for the Delivery of HACCP to the Food Service Sector

Project Leader:
Tom Abernathy, Ph.D.
Central West Planning Information Network
Hamilton

Collaborating Establishment(s):
Brant County Health Unit, Haldimand-Norfolk Regional Health Department, Halton Regional Health Department, City of Hamilton Social & Public Health Services Division, Regional Niagara Public Health Department, Waterloo Region Community Health Department, Wellington-Dufferin-Guelph Health Unit

Project Duration:
Nov. 2001 – Aug. 2003

Abstract:

Food-borne diseases are important contributors to human morbidity and mortality, and result in a significant cost to the health care sector. Recent changes in eating habits, and the tendency for people to eat more of their meals outside of the home, only increase the risk posed by these illnesses. One method for improving the operation of restaurants draws from the experience of the food processing industry. It involves the introduction of a system of procedures based on Hazard Analysis and the identification and monitoring of Critical Control Points (HACCP).

This project evaluated a program that applied the principles of HACCP to the food service industry. It successfully demonstrated that among a sub-population of restaurant operations such a program not only is acceptable, but capable of producing tangible results. Results also suggest that the principles and methods of the program (i.e., an initial assessment of the site, working with the operator to identify and suggest improvements, and return visits to monitor compliance) could be transferred to other types of food service operations.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Results of this project will be shared with public health units in the province, operators of food service premises, the provincial government and the public health community through presentations, reports and published articles.

This project will contribute to the food safety system in Ontario by evaluating how the application of HACCP principles can be applied successfully to the food service industry. The result will be an improved food safety intervention to benefit those at the local, provincial, federal levels of government as well as the food service industry itself.

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RM2: Evaluation of Essential Oils as an Alternative to Dietary Antibiotics to Control Food-borne Pathogens in Livestock

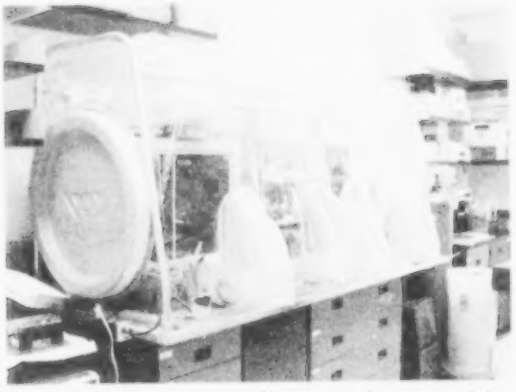
Abstract:

This study is to identify essential oils that can selectively inhibit the growth of *Salmonella* and *E. coli* O157:H7 *in vitro*. It will also examine the effects of identified essential oils on *Salmonella* and *E.coli* O157:H7, gut flora, gut health, and animal growth performance in vivo.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The success of this project would provide an alternative means to control *Salmonella* and *E. coli* O157:H7 in swine production. This could potentially reduce the risk of the industry on dietary antibiotics, minimize the development of antibiotic resistance and enhance Ontario food safety and quality.

Dr. Joshua Gong, Research Scientist at AAFC with research interests in alternatives to dietary antibiotic



Anaerobic chamber, oxygen-free atmosphere essential for culturing of gut bacteria and development of probiotics.

Project Leader:
Joshua Gong, Ph.D.
Agriculture and Agri-Food Canada

Collaborating Researcher(s):
R. Cao and T. Zhou, Food Research Program, AAFC; C. F. de Lange and R. Friendship, University of Guelph; C. Poppe and R. Johnson, LFZ, Health Canada, Guelph; W. Bo, OMAF

Project Duration:
Nov. 2002 - Dec. 2004

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RM3: Understanding Barriers to the Effective Implementation of HACCP in the Ontario Food Processing Sector

Project Leader:
Spencer Henson, Ph.D.
University of Guelph

Collaborating Researcher(s):
John Cranfield and Mansel Griffiths,
University of Guelph

Project Duration:
Jan. 2003 - Dec. 2004

Contact information:
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Abstract:

The proposal aims to improve understanding of the factors influencing adoption of HACCP by food processing enterprises in Ontario, and to identify effective strategies through which barriers to adoption can be alleviated, with particular emphasis on small and medium-sized enterprises that are non federally-registered. It seeks both to identify and quantify the factors motivating adoption of HACCP, the associated firm-level costs and benefits, and the barriers that can act to inhibit the implementation of HACCP. These are compared across sub-sectors of food processing and enterprise size.

The project consists of eight stages. Initially, a thorough review of previous studies on HACCP adoption and extensive consultation with key stakeholders and informants in Ontario are undertaken. The first stage of data collection involves a series (n=36) of case studies of food processing enterprises that have implemented HACCP, encompassing a range of sub-sectors and enterprises of differing size (including those that are not federally-registered). The case studies aim to produce in-depth information on the process of HACCP adoption, the associated firm-level costs and benefits and the barriers that need to be overcome. The second stage of data collection involves a postal survey (n=1,000) of food processing enterprises, aiming to both validate and quantify the results of the case studies and to provide an estimate of the numbers and types of food processing enterprises that have adopted HACCP in Ontario. Potential strategies through which the implementation of HACCP might be enhanced in Ontario are then developed through extensive consultation and a Delphi survey of key stakeholders and informants. These are then integrated into a framework that can be used by government agency and/or the food-processing sector directly to facilitate HACCP adoption.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The main outcomes of the project will be two-fold. Firstly, a better understanding of the process of HACCP implementation, the associated firm-level costs and benefits and the nature and extent of barriers that can inhibit implementation in the context of the food processing sector in Ontario. Secondly, a series of strategies through which barriers to the adoption of HACCP might be overcome in an effective and cost-efficient manner.

RM4: Evaluation of the Ability of Seaweed Extract (Tasco-14) to Reduce the Duration and Intensity of Fecal Shedding of Escherichia coli O157:H7 and Total E. coli by Cattle

Abstract:

A pre-harvest intervention strategy to reduce the duration and intensity of *E. coli* O157:H7 fecal shedding by cattle would prove to be instrumental in minimizing carcass contamination with *E. coli* O157:H7 and increasing the safety of Canadian beef. Acadian Seaplants Limited produce Tasco™ , a proprietary product processed from the seaweed *Ascophyllum nodosum*. *A. nosodum* is the most widely researched seaweed for agricultural purposes and is found growing along the coastline between high and low tides of the North Atlantic Ocean extending from Nova Scotia to Norway. Seaweeds have been fed to livestock throughout history and have GRAS (Generally Recognized as Safe) status with the FDA. A recent study showed that supplementation of Tasco - EX (extract from *A. nodosum*) to feedlot cattle two weeks prior to slaughter reduced *E. coli* (especially *E. coli* O157:H7) levels in fecal and hide samples (Behrends et al, 2000). More recently these same effects have been observed by incorporating Tasco -14™ at 2% of the ration (DM basis) for a period of 2 weeks immediately before slaughter (Barham et al., 2001; Evans, 2002).

The goal of this research is to evaluate the effects of Tasco-14 on the fecal shedding of *E. coli* O157:H7 by cattle through the use of controlled animal inoculation studies. A dietary intervention strategy, such as the incorporation of Tasco-14 into the diets of cattle two weeks prior to slaughter, would prove to be an economically feasible method to control *E. coli* O157:H7.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Pro-active steps must be taken to reduce the fecal shedding of *E. coli* O157:H7 by livestock. To date, there are no effective methods to control the fecal shedding of *E. coli* O157:H7 by cattle. A recent study showed that incorporating Tasco -14™ at 2% of the ration (DM basis) for a period of 2 weeks immediately before slaughter reduced levels of *E. coli* O157:H7 in the feces and on the hides of cattle (Barham et al., 2001; Evans, 2002). The present study is designed to evaluate the effectiveness of Tasco-14 in reducing the incidence of *E. coli* O157:H7 in experimentally inoculated cattle and may represent the identification of an economically feasible management strategy to control *E. coli* O157:H7 in cattle and thereby lower the associated risk of environmental contamination. Proactive approaches to controlling *E. coli* O157:H7 in beef cattle would represent a significant contribution towards minimizing the risk that this pathogen presently poses on the Canadian food supply.

Project Leader:
T. A. McAllister, Ph.D.
Agriculture and
Agri-Food Canada

Collaborating Researcher(s):
Yuxi Wang and Susan Bach, Agriculture and Agri-Food Canada

Project Duration:
Jan. 2004- Dec. 2005

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mcallister@agr.gc.ca

RM3: Understanding Barriers to the Effective Implementation of HACCP in the Ontario Food Processing Sector

Project Leader:

Collaborating Researcher(s):

Project Duration:

Contact information:

Abstract:

This proposal aims to improve understanding of the factors influencing adoption of HACCP by food processing enterprises in Ontario, and to identify effective strategies through which barriers to adoption can be alleviated, with particular emphasis on small and medium-sized enterprises that are non federally registered. It seeks both to identify and quantify the factors motivating adoption of HACCP, the associated firm-level costs and benefits, and the barriers that can act to inhibit the implementation of HACCP. These are compared across sub-sectors of food processing and enterprise size.

The project consists of eight stages. Initially, a thorough review of previous studies on HACCP adoption and extensive consultation with key stakeholders and informants in Ontario are undertaken. The first stage of data collection involves a series (n= 30) of case studies of food processing enterprises that have implemented HACCP and encompassing a range of sub-sectors and enterprises of differing size (including those that are not federally registered). The case studies aim to produce in-depth information on the process of HACCP adoption, the associated firm-level costs and benefits and the barriers that need to be overcome. The second stage of data collection involves a postal survey of c. 1000 of food processing enterprises, aiming to both validate and quantify the results of the case studies and to provide an estimate of the numbers and types of food processing enterprises that have adopted HACCP in Ontario. Potential strategies through which the implementation of HACCP might be enhanced in Ontario are then developed through extensive consultation and a Delphi survey of key stakeholders and informants. These are then integrated into a framework that can be used by government agency and/or the food processing sector directly to facilitate HACCP adoption.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The main outcomes of the project will be two-fold. Firstly, a better understanding of the process of HACCP implementation, the associated firm-level costs and benefits and the nature and extent of barriers that can inhibit implementation in the context of the food processing sector in Ontario. Secondly, a series of strategies through which barriers to the adoption of HACCP might be overcome in an effective and cost-efficient manner.

RM4: Evaluation of the Ability of Seaweed Extract (Tasco-14) to Reduce the Duration and Intensity of Fecal Shedding of Escherichia coli O157:H7 and Total E. coli by Cattle

Abstract:

A pre-harvest intervention strategy to reduce the duration and intensity of *E. coli* O157:H7 fecal shedding by cattle would prove to be instrumental in minimizing carcass contamination with *E. coli* O157:H7 and increasing the safety of Canadian beef. Acadian Seaplants Limited produce Tasco™, a proprietary product processed from the seaweed *Ascophyllum nodosum*. *A. nosodum* is the most widely researched seaweed for agricultural purposes and is found growing along the coastline between high and low tides of the North Atlantic Ocean extending from Nova Scotia to Norway. Seaweeds have been fed to livestock throughout history and have GRAS (Generally Recognized as Safe) status with the FDA. A recent study showed that supplementation of Tasco™ EX (extract from *A. nodosum*) to feedlot cattle two weeks prior to slaughter reduced *E. coli* (especially *E. coli* O157:H7) levels in fecal and hide samples (Behrends et al, 2000). More recently these same effects have been observed by incorporating Tasco™-14™ at 2% of the ration (DM basis) for a period of 2 weeks immediately before slaughter (Barham et al., 2001; Evans, 2002).

The goal of this research is to evaluate the effects of Tasco-14™ on the fecal shedding of *E. coli* O157:H7 by cattle through the use of controlled animal inoculation studies. A dietary intervention strategy, such as the incorporation of Tasco-14™ into the diets of cattle two weeks prior to slaughter, would prove to be an economically feasible method to control *E. coli* O157:H7.

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Project Leader:
T. A. McAllister, Ph.D.
Agriculture and Agri-Food Canada

Collaborating Researcher(s):
Yuxi Wang and Susan Bach, Agriculture and Agri-Food Canada

Project Duration:
Jan. 2001 - Dec. 2002

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RM5: Antibiotic Replacement Therapy for Control of Food-borne Pathogens in Poultry

Project Leader:
Shayan Sharif, Ph.D.
University of Guelph

Collaborating Researcher(s):
James Chambers and Joshua Gong, Food Research Program, AAFC;
Tony Hayes, University of Guelph;
Babak Sanei, OMAF

Project Duration:
Nov. 2003 - Oct. 2005

Abstract:

Food-borne pathogens, especially *Salmonella* and *Campylobacter*, cause serious illness in humans. These two pathogens account for over 90% of all food poisoning cases worldwide. Chickens and chicken products may harbor these pathogens and the use of antibiotics in chickens may result in the emergence of antibiotic-resistant bacteria in the gut. The main objective of this study is to manipulate the chicken gut microflora, via the use of prebiotics/probiotics, to effectively reduce *Salmonella* burden and shedding. Furthermore, this study seeks to determine the effects of these manipulations on the chicken immune system. Examination of immunological attributes of prebiotics/probiotics will provide a platform for discovering novel prophylactic strategies against infectious diseases in chickens. This is a collaborative and multi-disciplinary research program with scientists from University of Guelph, Agriculture and Agri-Food Canada, and Ontario Ministry of Agriculture and Food.

Expected Impact of Project Outcomes on Food Safety in Ontario:

Every year, there are over 17,000 reported cases of human infections with *Salmonella* and *Camplyobacter* in Canada. Poultry products are the source of infection in some of these cases. Moreover, consumers have growing concerns about food-borne pathogens, presence of antibiotic residues in food, and emergence of antibiotic-resistant microorganisms. With imminent restrictions on the subtherapeutic use of antibiotics in food animals and worldwide migration towards antibiotic replacements, it is apparent that alternative strategies should be sought to effectively address the above issues. The proposed study is designed to assess the feasibility of using antibiotic replacements, in form of pre/probiotics, in poultry feed to reduce shedding of food-borne pathogens. It is expected that the findings of this study will be directly applied to poultry production in Ontario and should significantly reduce the risk of presence of food-borne pathogens in poultry products. Moreover, these interventions will have an impact on poultry health, in general, thus reducing the need for other therapeutic and prophylactic interventions.

Contact information:
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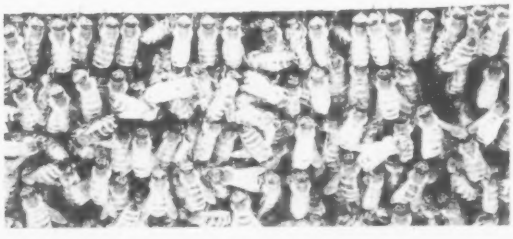
RM6: Enhancing the Food Safety of Honey Bee Hive Products Through the Use of Organic Beekeeping Practices and Effective Monitoring of Pests and Disease

Abstract:

Two deadly parasitic mites have drastically changed the beekeeping industry. These mites annually kill 50-70% of bee colonies, if not controlled. The use of miticides is problematic due to resistance development and the potential for residues in hive products. This is a pro-active project intended to keep pace with emerging residue issues in the bee industry.

Contamination of honey from mite treatment is a major concern. A recent problem involves the ban on antibiotic contaminated Chinese honey. In addition, coumaphos residues have been found in beeswax in the US. Honey in contact with coumaphos treated wax was found to extract the insecticide from the wax.

This project involves the use of organic beekeeping practices to manage honey bee colonies. Forty colonies will be established with mite resistant queens from tested Ontario bee stock. Cultural management techniques such as screened bottom boards, interruption of brood rearing and drone brood removal, will be evaluated. Treatments that will be applied to the colonies, formic acid and oxalic acid, are organic. No antibiotics or pesticides will be used. Parasitic mites and diseases will be thoroughly monitored. Bees will be dissected to determine tracheal mite infestation. Varroa mite levels will be monitored using a sticky board, an ether roll, a sugar dusting, an alcohol wash and by uncapping drone brood. Visual examination for American foulbrood (AFB) will take place on a regular basis, followed by microscopic confirmation.



Expected Impact of Project Outcomes on Food Safety in Ontario:

There is value in this work for conventional beekeepers, who will benefit from disease monitoring information and can work towards a decreased dependency on chemicals.

Results will lead to recommendations for organic management and disease monitoring methods, both of which will help to prevent contamination in hive products. The prevention of contamination issues in the bee industry will increase the marketability of our hive products. Reduction in miticide use will also decrease chemical contaminants in the environment. Healthy honey bee colonies, that are not dependant on miticides, will increase the number of colonies available for the pollination of Ontario's crops.

Clockwise from top: A nice, strong colony; One quarter cup of bees (photos by Alison Skinner); Bee cluster (photo by Janet Tam).

Project Leader:
Alison Skinner
Ontario Beekeepers' Association

Collaborating Researcher(s):
Paul Kelly, University of Guelph;
Janet Tam and Shannon Melin, Ontario Beekeepers' Association

Project Duration:
Oct. 2003 - Aug. 2005

Contact information:
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RM5: Antibiotic Replacement Therapy for Control of Food-borne Pathogens in Poultry

Project Leader:
Shayan Sharif, Ph.D.
University of Guelph

Collaborating Researcher(s):
James Chambers and Joshua Gong, Food Research Program, AAFC;
Tony Hayes, University of Guelph;
Babak Sanei, OMAF

Project Duration:
Nov. 2003 - Oct. 2005

Abstract:

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RM6: Enhancing the Food Safety of Honey Bee Hive Products Through the Use of Organic Beekeeping Practices and Effective Monitoring of Pests and Disease

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Project Leader:
Alison Skinner
Ontario Beekeepers Association

Collaborating Researcher(s):
Nick Kelly, University of Guelph
Janet Tam and Shannon Mans, Ontario Beekeepers Association

Project Duration:
Oct. 2003 - Aug. 2005

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RM7: Elimination of Human Pathogens on Seeds Destined for Sprout Production Using a Novel Sanitizer

Project Leader:
Keith Warriner, Ph.D.
University of Guelph

Collaborating Researcher(s):
M. Kostrzynska, Food Research Program, AAFC; M. Dixon and M. Griffiths, University of Guelph

Project Duration:
Jan. 2004 - Dec. 2004

Abstract:

Contaminated seeds have been implicated in the majority of foodborne illness cases associated with sprouts (bean sprouts, alfalfa). Despite intensive research no totally effective seed decontamination method has yet been developed. Indeed, it is acknowledged that the currently recommended 20,000 ppm calcium hypochlorite cannot guarantee inactivation of all pathogens present on seeds. We have developed a more effective method using a novel sanitizer (SDH). In initial laboratory based trials using mung beans inoculated with *Salmonella*, *E. coli* or *Listeria monocytogenes*, SDH treatment inactivated all pathogens with no detrimental loss in sprout quality. The current project aims to further evaluate SDH in decontaminating a diverse range of seeds inoculated with virulent strains of *E. coli* O157:H7 and *Salmonella*. The routes by which seed is contaminated in the field or during processing will also be investigated. Other aspects of the work will elucidate the mode of action of SDH and develop real-time PCR protocols for determining low levels of pathogens in sprouts/irrigation water.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The SDH treatment to be evaluated is reliable, requires no specialized equipment or training, uses concentrations in the order of 100 ppm, cost effective ($10 per ton of sprouts), contains an active ingredient approved for food use by the FDA and can be readily applied in the domestic or commercial environment. Rapid test methods exist to quantify the concentration of SDH and hence can be readily incorporated into a Hazard Analysis Critical Control Point (HACCP) plan.

The nutritional benefit of sprouts is well established but due to the association with virulent pathogens many high risk groups (young, elderly) have been advised against consuming such products. Obviously by providing pathogen-free sprouts the health benefits derived from sprouted seeds can be realized. As the United States market is currently contracting due to the food safety issues ($50M loss in 1999) the prospect of increased exports from Ontario is a real possibility.

Rajnessh Hora (Research Assistant, UofG) who is working on this project.

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RM8: Practical Optimization of Ozonation Process for Enhancing Microbial Safety and Food Quality

Abstract:

Risks from microbial contamination and the potential to form toxic by-products during disinfection with chlorinated compounds are concerns in the food industry. Ozone, being widely used as an alternative disinfectant to chlorine in water treatment, has been demonstrated to be a promising technology, which could be used in food industry to ensure microbial safety and quality of foods. It has powerful disinfecting ability, high reactivity and spontaneously decomposes to non-toxic oxygen. To properly apply this technology in practice, research is needed to quantify the effectiveness of ozonation for different food products under different scenarios and provide the necessary design protocols. Thus, the research objectives of this project include:

- To expand the knowledge of ozone application for food processing industry by using fresh strawberry and green pepper grown with *E. coli* O157:H7, *Listeria* spp. and *Salmonella* spp. respectively.
- To quantify the inactivation kinetics for different bacterial contaminants under more realistic conditions by varying bacterial growth conditions.
- To conduct semi-batch tests so that the results from the laboratory batch testing can be compared and verified further.
- To evaluate different dissolution technologies that can be used for food processing.
- To modify and verify the applicability of the integrated ozonation process model developed by the principal investigator for food processing.
- To provide the regulatory and design guide lines that can achieve effective controlling of microbial risks while maintaining the economic competitiveness.

Expected Impact of Project Outcomes on Food Safety in Ontario:

The results of this research may provide Ontario food processors with a new preservation technology based on the use of ozone as a disinfectant. As well, the effectiveness, limitations and operating protocols of ozone application in food applications will be summarized. The technology also has the potential to reduce wastewater generation via processing water reuse.

Project Leader:
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Collaborating Researcher(s):
T. Zhou and R. McKellar, Agriculture and Agri-Food Canada

Project Duration:
Sept. 2002 - Sept. 2004

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RM7: Elimination of Human Pathogens on Seeds Destined for Sprout Production Using a Novel Sanitizer

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

Rajeesh Rava (Research Assistant, UofG) who is working on this project.

RM8: Practical Optimization of Ozonation Process for Enhancing Microbial Safety and Food Quality

Abstract:

Expected Impact of Project Outcomes on Food Safety in Ontario:

SECTION THREE: Quick Search

Subject:	Abstract:
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